obtained on ascites fluids[1,2] or on grafted tumours[3] are comparable to the conditions present in the tumour of origin.

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Effect of Bacillus Calmette-Guérin Infection on Transplanted Tumours in the Mouse

During the growth of certain transplanted tumours considerable hyperactivity of the reticulo-endothelial system is observed.[1] Similar alterations are also found in the first stage of experimental infections[2], suggesting that the host response to foreign tissue and some infectious agents is closely related. Agents such as endotoxins, zymosan, products of the tubercle bacillus, and Bacillus Calmette-Guérin infection which enhance the activity of the reticulo-endothelial system[3] and the capacity for antibody production[4] also increase natural resistance to infection[5]. In light of these observations, we have attempted to alter the growth and lethality of various experimental tumours by agents known to possess the common property of stimulating the phagocytic capacity of the reticulo-endothelial system. One such agent, zymosan, has been demonstrated to increase significantly the regression rate of the mouse tumour, sarcoma 180 (*S*-180), under certain conditions[6]. The present report deals with the course of three transplantable tumours, *S*-180, carcinoma 755 (*Ca* 755), and Ehrlich ascites, in mice infected with Bacillus Calmette-Guérin.

Young, female *Ha/ICR* Swiss mice and *C*57 hybrid mice (bred by Dr. J. J. Bittner, University of Minnesota) weighing approximately 18–20 gm., were injected intravenously with one mgm. Bacillus Calemtte Guérin wet weight. The Bacillus Calmette-Guérin (Phipps' strain) was grown in either Sauton's (supplied through the courtesy of Mr. H. J. Henderson, Phipps Inst., Phila.,Pa) or the Dubos' liquid medium. Neither morbidity nor mortality attributable to Bacillus Calmette-Guérin infection alone was observed; infected animals appeared active and perfectly healthy. At selected intervals following Bacillus Calmette-Guérin inoculation, infected animals and appropriate controls were challenged with either solid tumour (*S*-180, *Ca* 755) implanted subcutaneously or by intraperitoneal injection of Ehrlich ascites cells.

Growth of *S*-180 in normal *Ha/ICR* Swiss mice is characterized by death of 85–90 per cent of hosts in two to five weeks; the remainder of the mice undergo spontaneous regression of their tumours. The effect of Bacillus Calmette-Guérin infection on the mortality associated with growth of this tumour is presented in Table 1. In mice implanted with *S*-180 one day

Table 1. Mortality following *S*-180 Implantation.

	Days between B.C.G. infection and tumour inoculation					
Controls ..	1	7	14	19	25	67
68/79† ..	13/15	3/12	0/12	9/30	0/8	0/9

† mortality/number per group.

following infection the regression rate was normal, whereas mice inoculated with the tumour seven days, or longer, after Bacillus Calmette-Guérin infection showed definite protection. Of the groups at seven and nineteen days post-infection, 70–75 per cent of tumours regressed; mice inoculated with *S*-180 fourteen, twenty-five, and sixty-seven days following Bacillus Calmette-Guérin infection were completely resistant to tumour growth. The tumours in Bacillus Calmette-Guérin infected animals developed normally for the first seven to ten days and then began decreasing in size after the second week. The process of regression in animals infected with Bacillus Calmette-Guérin was essentially similar to that observed in the few control mice which rejected their tumours. In a group of *C*57 hybrid mice implanted with *S*-180 fourteen days following Bacillus Calmette-Guérin infection, only one out of eight animals regressed the implanted tumour; none of the tumours in the control animals regressed. The finding that *C*57 hybrid mice responded poorly to *S*-180 inoculation at a time when Swiss mice were completely protected correlates well with our unpublished observation that the *C*57 hybrid does not attain as high a degree of reticulo-endothelial stimulation as Swiss mice following Bacillus Calmette-Guérin infection.

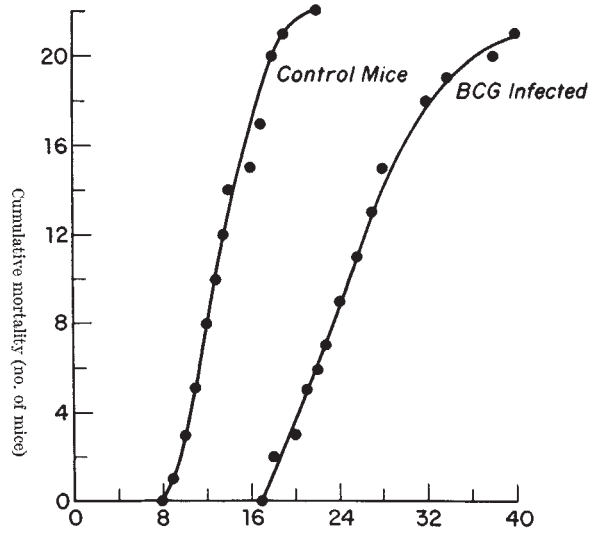

Fig. 1.

Fig. 1 demonstrates the altered course of the Ehrlich ascites tumour in Bacillus Calmette-Guérin infected Swiss mice. The average survival time in uninfected controls was 14 days. In animals inoculated with Bacillus Calmette-Guérin eleven or thirteen days previously, it was 27 days. Ascites formation in infected animals was not inhibited; in fact, during the course of the enhanced survival time, these animals frequently developed hugely distended abdomens. Despite the presence of appreciable quantities of ascitic fluid, the infected animals remained healthy and active for a longer period than their corresponding controls.

Table 2. *Ca* 755 IMPLANTED 17 DAYS FOLLOWING *Bacillus Calmette-Guérin* INFECTION IN *C*57 HYBRIDS.

	Average tumour diameter (cm.)		
	13 Controls	10 B.C.G. Infected	Per cent mortality B.C.G./Controls
25 days ..	2·04	0·82	0/0
33 days ..	2·68	1·21	0/23
48 days ..	3·56	2·39	0/92

B.C.G.—*Bacillus Calmette-Guérin.*

Table 2 summarizes the results of experiments concerned with the growth of *Ca* 755 in both normal and Bacillus Calmette-Guérin infected *C*57 hybrid mice. In addition to the slower growth of this tumour, the Bacillus Calmette-Guérin infected mice lived significantly longer and frequently showed advanced-to-complete regressive changes in their tumours prior to death. This retardation in *Ca* 755 growth and increased survival time has also been observed in mice of a *C*57 inbred line following Bacillus Calmette-Guérin infection.

The beneficial effect of Bacillus Calmette-Guérin infection on the outcome of *S*-180 growth appears most likely to be an expression of a more vigorous or accelerated homograft reaction. Most important, perhaps, is the finding that the mice are still resistant to the growth of *S*-180 sixty-seven days following infection. The significant degree of protection to *Ca* 755 in terms of tumour retardation and prolonged survival time also points to a more competent immune response in the infected host. The increased survival time in infected animals with Ehrlich ascites may reflect an enhanced, though insufficient, antibody response to the inoculated cells: however the results obtained may also be ascribed to a more efficient reaction to endogenous infection which frequently appears to be a contributing factor in the death of tumour bearers.

The studies reported herein have been exclusively concerned with transplanted tumours. Present experiments are in progress to extend these observations to the behaviour of first and second transplant generations of spontaneous tumours in Bacillus Calmette-Guérin infected isologous hosts. If some resistance based on an immunological response exists to the development and progression of spontaneous neoplasms, the Bacillus Calmette-Guérin infected host with its greatly enhanced capacity to respond to antigenic stimulation deserves special attention in studies concerning tumour immunity.

We wish to gratefully acknowledge the assistance of Miss M. Goldsmith. This work was aided by a grant from the American Cancer Society.

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MICROBIOLOGY

Lowered Bactericidal Efficiency of Hydrogen Peroxide on Milk from Cows treated with Penicillin

DURING the course of experiments on the introduction of hydrogen peroxide as a routine means of raw milk preservation, the following anomaly was observed.

In a certain number of trials, the usual rate of bactericidal efficiency, which normally fluctuated between 80 and 94 per cent of chemically pure 30 per cent hydrogen peroxide used at a concentration of 0·2 per cent was considerably lowered, at times by 30–70 per cent. In certain extreme cases, after one hour of hydrogen peroxide treatment, an actual rise in the initial number of milk microflora population occurred. At the same time many raw milk samples with a high catalase content were examined, where the percentage of destruction by hydrogen peroxide was lowered to 75 per cent of normal compared. It was therefore concluded that the anomalous results were caused by an unknown substance, present in raw milk.

The period of the investigation coincided with the summer of mass antibiotic treatment of the cattle, so we investigated the possibility that antibiotics in the milk were the cause of the interference phenomenon. Large number of milk samples containing penicillin were treated with hydrogen perioxide. The results were consistent with the supposition that the interfering substance was penicillin, which had been secreted into the milk during and after the treatment of the cows. Total counts of milk by the pour plate method were made on Difco tryptone glucose yeast agar, and the observed results revealed significant differences in the percentage of destruction, as compared with those of normal raw milk.

Mixtures of 0·1, 0·5, 1·0, 5·0, 10, 20, I.U./ml. penicillin (crystalline sodium *G*) with 0·2 per cent of 30 per cent hydrogen peroxide in distilled water, and raw milk did not decompose hydrogen peroxide directly,