TABLE I. Blood Plasma Adenosine Deaminase of Normal Rats and of Rats with Fibrosarcoma.*

	Method of Koehler & Benz(5)		Method of Kalckar(6)	
	Controls	Fibrosarcoma	Controls	Fibrosarcoma
No. of rats	8	9	8	9
Range in units per 1 ml	3.9 — 12.8	20.6 — 46.7	11 — 38	27 — 150
$\bar{x} \pm S_{\bar{x}}$† units/1 ml	7.9 ± 1.0	34.4 ± 3.1	23 ± 4	74 ± 12
Significance of the differences	$t = 8.030$	$P < .001$	$t = 4.048$	$P < .01$

* Values expressed in units/1 ml.
† $\bar{x}$ = arithmetic mean.

$S_{\bar{x}}$ = Standard deviation of mean = $\pm \sqrt{\frac{(x - \bar{x})^2}{n\,(n-1)}}$

representing 5% of body weight, the enzyme showed normal values. These observations indicate that the increase of adenosine deaminase in plasma is not a precocious occurrence.

Summary. The levels of plasma adenosine deaminase were studied in rats with a transplantable fibrosarcoma and compared with normal rats. High values were found in the plasma of fibrosarcoma rats, the enzyme being estimated by two methods based on the ammonia liberated and measured colorimetrically, and on the decrease of the adenosine determined spectrophotometrically.

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Received September 24, 1962. P.S.E.B.M., 1962, v111.

Effect of BCG Infection on Leukemia and Polyoma in Mice and Hamsters.* (27908)

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Phagocytic activity and antibody production appear to be involved in the host response to neoplastic processes. Tubercle bacilli of the BCG strain have a stimulating effect on both functions and could thus exert an influence on neoplasms. BCG action was studied in several forms of tumors and found to be useful against some(1,2,3,4). This paper describes experiments in which the effect of BCG was investigated on transplanted and spontaneous leukemia in mice and on polyoma in hamsters.

Transplanted leukemia. Methods. Two experiments were carried out with transplanted leukemia. In the first, the transplanted tissue happened to be very active, killing controls in 15 days, and consequently the differences between BCG-treated mice and controls were not too conspicuous and significant. Therefore, only the second experiment will be reported in detail.

The experimental arrangement was essen-

* Aided by grant from Nat. Cancer Inst. of Canada.

TABLE I. Effect of BCG on Transplanted Leukemia.

Group	Body wt increase 16 days after implant. (g)	Survival time after implant. (avg days)	Tumor size at autopsy (length × width × thickn., mm)	Wt of lymphatic organs (mg/100 g body wt)		
				Spleen	Thymus	Lymph nodes
Live BCG	1.7 ± .2*	29.6 ± 1.4*	494 ± 127*	2090	665	2972
Controls	.1 ± .3*†	19.6 ± .8*†	847 ± 76*‡	1712	408	1949

* Std. error of mean. † Signif. difference, $P < 0.001$. ‡ *Idem*, $P < 0.05$.

tially the same in both experiments, except that, in the second, a smaller amount of tissue was transplanted, with a view to producing a slower disease and allowing BCG more chance to show possible antagonistic action. Forty mice, averaging 84 days of age and 24 g body weight, were divided into 4 groups of 10. They were inbred animals of the Ak leukemic line, descendants of breeders kindly given us by Dr. L. Gross in 1955. Sexes were distributed equally, litter mates as equally as possible, among the groups. Animals of the first group were inoculated with live BCG. Lyophilized BCG was used. Bacilli were grown on Sauton's medium, harvested, suspended in 15% lactose and lyophilized. Injections were made with this material resuspended in distilled water (1 mg wet weight in 0.25 ml i.v. to each mouse). The other groups served as controls: the second received heat-killed bacilli (56°C, 2 hr); animals of the third group were injected with 15% lactose; mice of the fourth group were left uninoculated. Three weeks later (5 in the second experiment), all animals underwent subcutaneous implantation of lymphatic tissue (spleen, thymus, lymph nodes, liver) pooled from 4 Ak leukemic mice; each animal received 0.3 ml of a 20% suspension in saline in the first experiment and 0.2 ml in the second.

Results. In the first experiment, no difference could be noted between groups as regards clinical appearance or take of implant. However, body weight continued to increase in the first group, whereas it remained stationary in the controls, the difference being significant. The size of the local tumor, at the site of implantation, was slightly smaller in the first group after 10 days, indicating that its growth tended to lag with BCG. Survival time was somewhat longer in the BCG group. At autopsy, local tumors showed no consistent group differences; however, the smallest was in the first group and the largest in the untreated controls. Spleen was most enlarged in the group treated with live BCG. This was not unexpected: BCG induces splenomegaly, as do most infections. Thus, spleen enlargement may be due to both BCG and leukemia. The thymus, affected only by leukemia, was smaller in the BCG group. Changes in the superficial lymph nodes were like those in the spleen, whereas in visceral nodes (mesenteric, renal, iliac) they resembled those in the thymus. The difference between the first and the control groups is significant, as far as thymus and visceral nodes are concerned.

In the second experiment, a difference was seen in the general condition: all controls soon began to look ill, at a time when BCG-pretreated mice still looked healthy and well. This is reflected in the body weight. For about 10 days after implantation, body weight progressed normally in all groups. Then it continued to increase in the BCG mice, but decreased in the controls. In Table I, the results of all control groups are combined, for simplicity, since no significant variations could be observed among them. With regard to the growth of local tumors, no clear-cut differences could be detected until the 20th day. Palpation of superficial lymph nodes did not reveal either any consistent variation between the groups. However, survival time was significantly longer in BCG-treated mice than in any of the control groups; death did not occur in the former until almost 80% of the controls were already dead. In addition, autopsy showed the local tumor to be considerably smaller in the first

TABLE II. Effect of BCG on Spontaneous Leukemia.

Group	No. leukemia cases			Incidence of leukemia %	Age at death (avg days)			
	Frank	Doubtful	Negative		All cases	When leukemia was: Frank	Doubtful	Absent
Live BCG	8	6	5	42-74	372 ± 24.8*	314	406	423
Controls	17	2	1	85-95	293 ± 18.6*†	298	311	153

* Std. error of mean.

† Signif. difference, $P < 0.02$.

group. Lymphatic organs were weighed and all found to be more enlarged in the same group. In other words, the subcutaneous tumor, as well as lymphatic organs, developed at about the same rate in all groups for 20 days. Then, in BCG-infected animals, during the prolonged survival period, the tumor began decreasing in size, to almost nothing in some individuals, but generalization of leukemia was not inhibited; in fact, these animals developed more enlarged lymphatic organs than did the controls. Therefore, whereas controls died of both the tumor and generalizing leukemia, BCG mice experienced a regression of their tumor and died, later, of generalized leukemia.

It may be noted that no significant effect of killed BCG was observed in these experiments.

Spontaneous leukemia. Methods. Twenty Ak mice, 12 males and 8 females, average age 73 days, were inoculated with live BCG, as previously described. Twenty litter mate controls (12 males, 8 females) received an injection of lactose solution. All animals were observed until spontaneous death. One died accidentally in the first group and is not included in the results.

Results. (Table II). The incidence of leukemia was much lower in the BCG group, and survival was significantly longer. The number of frank leukemia cases was decreased by half; non-leukemic and doubtful cases were more numerous. The latter consist of animals that died either with dubious symptoms of leukemia, or with leukemia and some other disorder. Two percentages are given for leukemia, depending on whether doubtful cases are counted as leukemic or not. Table II shows that, after BCG, leukemia being postponed and its incidence decreased, mice died later of other disorders (usually kidney lesions). The incidence of leukemia and survival time in the controls compare well with those in another earlier experiment, where they were 85% and 306 days respectively; in our Ak mouse colony, incidence is currently 86% and life span averages around 300 days.

Polyoma. Methods. Three-day-old hamsters were inoculated subcutaneously with 0.5 mg of live BCG. They were random-bred animals, progeny of breeders obtained from Bio-Research Consultants, Cambridge, Mass. Seven days later, they received an intraperitoneal injection of polyoma virus. The strain of polyoma used was isolated from Ak leukemic mice in mouse embryo tissue cultures. Supernatant fluids were pooled from a third passage and kept frozen at −60°C for 20 days before being administered (0.4 ml of fluid per animal). A control group of the same age received only the virus.

Results. A few sucklings died early and were devoured by their mothers, as is customary with young animals, especially hamsters. Inoculation of BCG or polyoma may have stimulated these deaths. It should be recalled that BCG is relatively noxious in hamsters(5). All other animals, except one, died of typical polyoma lesions: hemorrhagic blebs in viscera, multiple tumors. But the appearance of polyoma was delayed and consequent survival significantly prolonged in the BCG group (Table III). The latter animals also presented pathologic changes due to mycobacterial infection: nodules in the skin, at the site of BCG injection; tuberculoid foci in the lungs, liver, spleen, etc., with "corps concentriques" described long ago by Metchnikoff(7) and characteristic of this infection in hamsters. The one animal that had no evidence of polyoma belonged to this

TABLE III. Effect of BCG on Polyoma in Hamsters.

Group	No. hamsters: At beginning	No. hamsters: Died early and not considered in results	Survival age (avg days)
Live BCG	13	5	130.1 ± 26.1*
Controls	12	0	65.0 ± 9.2*†

* Std. error of mean.
† Signif. difference, $P < 0.03$.

group and apparently died of tuberculous infection.

Discussion. In brief, BCG was observed to afford protection against neoplastic conditions: better maintenance of body weight, regression of local tumor in transplanted leukemia, lower incidence of spontaneous leukemia, delayed development of polyoma, prolongation of survival time in all cases. A retardation of spontaneous leukemia in Ak mice with BCG was previously reported(3); the present experiments confirm this finding.

The mechanism of such protective effect may be, as was suggested(1,3,4), an enhancement of phagocytic activity of the reticuloendothelial system, or of immune responses, directed against primary tumor tissue, or against metastatic cells, considered as foreign material, or against oncogenic viruses. Other factors may come into play; for instance, the mild disturbance caused by BCG infection could increase the secretion of corticosterone or similar hormones, which may in turn exert an inhibiting action against neoplasms.

According to a long tradition, which may be traced back to Rokitansky(6), there is antagonism between tuberculosis and cancer. Several observations may be presented in favor of this opinion, but the evidence and views are conflicting: perhaps as many facts may be cited against as for this theory. Nevertheless, some interesting points call for consideration. For example, the decrease in death rate from tuberculosis is balanced by an increase of the death rate from malignancy, so that the joint rates remain relatively constant from year to year in several countries(8); this is perhaps more than fortuitous and suggests a causal relationship. A comparison of the figures for countries with high and countries with low death rates from tuberculosis in relation to death rates from cancer might give some indications also, but it is hardly possible to deduce very much from official statistics, because the manner in which data are collected varies considerably from one country to another and because many factors, such as nutrition, social conditions, etc., must be taken into account. A special study, with these points in view, should be undertaken, before reliable conclusions can be reached. One may ask what happens in countries where BCG has been administered for some time as a preventive measure against tuberculosis, but it is still too early for a definite answer: BCG has not been used on a large scale long enough to exert an appreciable influence on neoplastic diseases.

Summary. In Ak mice inoculated with live BCG and submitted to isologous transplantation of leukemic tissue, body weight was maintained longer, partial regression of local implanted tumors was observed, development of leukemia was delayed and survival prolonged, as compared with controls without BCG infection. In Ak mice similarly treated with BCG, the incidence of spontaneous leukemia was decreased and death was significantly retarded. In hamsters inoculated with live BCG and subsequently with polyoma virus, development of polyoma was delayed and survival considerably prolonged.

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