phosphodiesterase[10,11] and show that the increased susceptibility to cyclic AMP in contrast to dibutyryl cyclic AMP is not necessarily the property of tumorigenic cell lines as reported by others[5]. Figure 1 shows that the rate of RNA synthesis decreased significantly at 4 to 6 h, while the decrease in protein synthesis was not demonstrable until later at 8 h (Figure 2). Differences in the rate of DNA synthesis occurred only after 8 h. However, repeated experiments indicate consistently that the early decrease in the rate of DNA synthesis, starting within 1 to 6 h, in the treated cultures when compared to controls is real suggesting that the cells entering into the DNA synthesizing S phase of the cell cycle are preferentially inhibited. The rates of DNA synthesis expressed in terms of 10^6 viable cells clearly confirm that this is the case. Under the experimental conditions used, the cells showed a semisynchronous wave at 8 h. Selective inhibition at the site or stage of the initiation of DNA replication and RNA transcription seems to be the earliest biosynthetic mechanisms affected by cyclic AMP, substantiating the findings of LANGAN[12] and PASTAN, PERLMAN et al.[13]. LANGAN shows that histones serve as substrate in vivo for the cyclic AMP-dependent protein kinase and postulates that increased histone phosphorylation brought about by hormone administration may provide a mechanism for induction of RNA and protein synthesis in target tissue. PASTAN, PERLMAN et al.[13] have shown that stimulation of β-galactosidase synthesis by cyclic AMP occurs at the level of transcription of RNA and that the lac operon promotor is the site of action of the cyclic AMP. The different responses, stimulation or inhibition, of a cell towards stimuli may depend on the physiological and differential state of the cells as shown in other systems, e.g., responses of lymphocytes and lymphoblasts toward anticellular antibodies[6,14], phytohemagglutinin[15,16], and cyclic AMP[17]. Finally the decrease in the rate of DNA synthesis in the cyclic AMP treated cells is unlikely to be caused by activation of thymidine phosphorylase thus breaking down thymidine to thymine. RABINOWITZ and WILHITE[18] have shown recently that cyclic AMP did not alter the enzymes in thymidine salvage pathway in either normal or leukemic leukocytes[19].

Résumé. La croissance des cellules leucémie murine L-5178-Y-R gène le cycle AMP dans les cultures. Le taux des syntèses décroit d'une manière significative après 4 et 6 h pour le RNA et après 8 h pour la protéine. Le taux de la syntèse de DNA décroit quelque peu entre 1 et 6 h et d'une façon accentuée après 8 h.

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Increased Survival Time of Leukemic Mice Following Treatment with Phytohemagglutinin

Since the studies of NOWELL[1], the stimulation of mitosis of lymphoid cells in vitro by phytohemagglutinin (PHA) has been the subject of many investigations[2–5]. Recently, there has been increased interest in the effect of PHA on the immune response, both cell-mediated and humoral[6]. The evidence to date suggests that PHA depresses or enhances the immune response depending on the antigen, animal system, or the specific response studied[7–10].

The studies to be reported here concern the effect of PHA on the survival time of mice inoculated with the transplantable lymphocytic leukemia L1210.

Methods. BDF_1 (C57B1/6xDBA/2) male mice weighing 18–22 g were used for all studies. Phytohemagglutinin-P (PHA) was obtained from Difco Laboratories, Detroit, Michigan. The lypholized PHA was rehydrated with 5 ml of sterile saline and dosages are reported as volumes of the diluted material. The L1210 leukemia cells were harvested from the ascitic fluid of DBA/2 mice and diluted to 10^5 cells per 0.1 ml.

The first study was designed to determine the dose response relationship of PHA on the survival time of leukemic mice. 3 groups of 10 mice each were treated i.p. with 0.3, 0.2, or 0.1 ml of PHA. An additional 10 mice received 0.1 ml physiological saline and served as controls. PHA treatment was begun on day 1 and continued until death of the animal. On day 4 all mice received 10^5 L1210 cells i.p. The animals were observed daily for death and survivors were weighed to monitor signs of toxicity.

A second study using 3 groups of 5 mice each was designed to determine if pretreatment of the animals with PHA before L1210 implant was necessary in order to obtain an increase in survival time. One group was treated daily with 0.1 ml PHA from day 1 until death. A second group was treated daily with 0.1 ml PHA from

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day 5 until death and a third group, serving as controls, received 0.1 ml saline daily from day 1 until death. All animals were implanted with 10^5 L1210 cells on day 4 and observed daily for deaths.

The third study in this series was conducted to examine the antileukemic effectiveness of PHA and 6-Mercaptopurine (6-MP) alone and in combination. 6-MP is known to be effective against the L1210 tumor system and acts by interfering with the nucleic acid synthesis. 40 mice were divided into 4 groups of 10 mice each and 1 group was treated daily with 0.1 ml PHA from day 1 to death. A second group received 20 mg/kg/day of 6-MP from day 5 to death. The third group was treated with 0.1 ml PHA from day 1 to death and with 20 mg/kg/day of 6-MP from day 5 until death. The fourth group served as controls and were treated with 0.1 ml saline from day 1 until death. All animals were implanted on day 4 with 10^5 L1210 leukemia cells and were observed daily for deaths.

The fourth study was designed to examine the antileukemic activity of PHA in animals which were implanted with 10^3, 10^4, 10^5, or 10^6 L1210 cells. 40 mice were divided into 8 groups of 5 mice each and 4 of the groups were treated with 0.1 ml PHA from day 1 to death. The remaining 4 groups received 0.1 ml saline from day 1 to death. On day 4 one each of the treated and nontreated groups were implanted with 10^3, 10^4, 10^5, or 10^6 L1210 cells. The animals were observed daily for deaths.

Results. Toxicity as judged by increasing lethargy and roughened coat, evident by day 3, was encountered with a PHA dose of 0.3 ml per day, and by day 4, four animals had died. There was no weight loss associated with the observed toxicity.

The mice receiving 0.2 ml and 0.1 ml of PHA had an increase in mean survival time over that obtained in the controls. Daily treatment with 0.2 ml PHA resulted in a 40% increase in life span and 0.1 ml gave a 51% increase (Table I). These increases were both significant, $P \leq 0.01$, as determined by Student's *t*-test.

Pretreatment with 0.1 ml PHA resulted in a 25% increase in life span in the second study but no increase in life span was obtained when PHA treatment was begun 24 h after tumor implant (Table II).

Treatment with 0.1 ml PHA beginning on day 1 or with 6-MP, 20 mg/kg/day beginning on day 5 resulted in comparable increases of survival time, 39% and 42% respectively. The combination of PHA and 6-MP resulted in a 65% increase in survival time over the controls. This increase in survival time over that produced by either treatment alone is statistically significant, $P \leq 0.01$, and suggest a synergistic action (Table III).

PHA treatment was more effective in increasing the life span of animals that received 10^6 L1210 cells than in those that received 10^5 or 10^4 cells (Table IV). There was no increase in survival time of the animals that received 10^3 leukemic cells. The mean survival times of the PHA treated animals were similar regardless of the leukemic cell inoculum. The difference in survival times was due to the earlier deaths resulting from increased leukemic cell inoculum.

Discussion. These studies demonstrate that PHA is effective in increasing the survival time of leukemic mice. The results obtained could be explained as a direct effect of PHA on the leukemic cells or as a preconditioning of the animals' immune response in a manner that exerts an inhibitory action upon the leukemic cells. An enhancement of the immune response, as shown by SINGHAL et al.[11], is suggested by the pretreatment study where PHA was effective if given before leukemic cell inoculum, but had no effect when given 24 h after tumor implant. Suppression of the immune response as has been shown

Table I. Effect of PHA-P on survival time of L1210 leukemic mice

Treatment	MST[a] (days)	ILS[b] (%)
PHA-P 0.3 ml	–	0
PHA-P 0.2 ml	12.6	40
PHA-P 0.1 ml	13.6	51
Saline 0.1 ml	9.0	–

[a] Mean survival time. [b] Increase in life span.

Table II. Effect of daily PHA-P administration on survival time of L1210 leukemic mice when treatment is begun 3 days prior to L1210 implant versus treatment beginning 24 h after L1210 implant

Daily treatment	MST[a] (days)	ILS[b] (%)
0.1 ml PHA-P, Pre-implant	11.0	25
0.1 ml PHA-P, Post-implant	9.0	2
0.1 ml Saline	8.8	–

[a] Mean survival time. [b] Increase in life span.

Table III. Effect of PHA-P and 6-mercaptopurine on the survival time of L1210 leukemic mice

Daily treatment	MST[a] (days)	ILS[b] (%)
0.1 ml PHA-P days 1-death	12.3	39
6-MP[c], 20 mg/kg days 5-death	12.5	42
6-MP, 20 mg/kg and 0.1 ml PHA-P days 5-death and 1-death respectively	14.5	65
0.1 ml Saline days 1-death	8.8	–

[a] Mean survival time. [b] Increase in life span. [c] 6-Mercaptopurine.

Table IV. Effect of PHA on survival time of mice implanted with varying numbers of leukemic cells

Leukemic cell inoculum	PHA treated MST[a] (days)	No PHA treatment MST (days)	ILS[b] (%)
10^3	13.2	13.0	1
10^4	12.6	11.0	14
10^5	13.2	9.6	37
10^6	11.0	7.6	44

[a] Mean survival time. [b] Increased life span.

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by ST. PIERRE[7] and ST. PIERRE et al.[8] and STEFANI and MOORE[12] would result in a diminished survival time.

Evidence for a direct action of PHA on the leukemic cells is provided by the results obtained from the study in which the number of cells inoculated was varied. With the smallest inoculum (10^3 cells), PHA treatment had no effect on survival time but with 10^6 cells, PHA increased survival time by 44%. Studies to be reported at a later date have shown that L1210 leukemic cells are agglutinated in vivo by PHA. A proposed mechanism could be that a critical number of cells is needed within a certain volume before the agglutinating action of PHA is expressed. Agglutination would inhibit the uptake of the leukemic cells by the blood stream and lymphatics and reduce the number which eventually lodge within the filtering organs. Agglutination may also inhibit the metabolic activity of the leukemic cells resulting in a reduced mitotic level.

At the present time it is not clear whether PHA affects the animal or the leukemic cells but it is evident that leukemic animals treated with PHA survive longer than the untreated controls. The mechanism of this effect is being investigated with a combination of in vivo and in vitro studies[13].

Résumé. Des souris ayant subi un traitement journalier au PHA, entrepris 4 jours avant l'implantation de cellules leucémiques L1210 on survécu plus longtemps que celles qui furent traitées au sel. Le traitement journalier entrepris 24 h après l'inoculation de cellules leucémiques n'a pas eu d'effet sur la durée de survie. Par le traitement au PHA combiné à la 6-mercaptopurine, la durée de vie fut plus longue que par celui que l'on obtint avec d'autres agents employés isolément. Un mécanisme proposé pour l'effet antileucémique de PHA est discuté.

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Active Immunotherapy of Mouse RC 19 and E ♀ K1 Leukaemias Applied After the Intravenous Transplantation of the Tumour Cells[1]

In a series of experiments carried out on L 1210 leukaemia grafted subcutaneously, we showed that active immunotherapy can be effective, delaying and reducing the mortality, even when it is applied after the graft of the tumor cells[2,3]; the necessary condition of its efficiency was that the number of tumor cells be $\leq 10^5$.

In these experiments, the living tumour cells were grafted subcutaneously in such a way that the tumour volume could be measured and the effect of the immunotherapy on the tumour growth could be quantitatively estimated. Nevertheless, it is questionable whether the effect of active immunotherapy might not be due to the stimulation of a lymph node not yet immunologically informed at the time of the immunological stimulation by the tumour associated antigen, and if the result obtained could be found again in human leukaemia, which is, at least at the time of treatment, a disseminated neoplasia. Another criticism which could be made of our L 1210 leukaemia experiments concerns the history of this grafted tumour which has been transmitted by transplantation through many generations, and of which the tumour associated antigens are difficult to demonstrate[4].

Hence the idea of treating with active immunotherapy other experimental mouse leukaemias, the cells of which are 1. carrying tumour associated antigens which are well precized; 2. disseminated because inoculated intravenously.

Material and methods. 1. *Leukaemia RC 19.* 60 (DBA/2 × Balb/c) F1 female mice, aged 3 months, received by intravenous route 10^3 cells from (Rauscher) leukaemia RC 19, which is transmissible by graft. These animals were divided at random into 4 groups: group a) comprised 15 controls; group b) 15 mice which received 24 h after the leukaemia graft 1 mg living B.C.G.[5] intraperitoneally, this injection being repeated every 4 days for 16 days; group c) comprised 15 mice which received 48 h after the graft of the leukaemia a peritoneal injection of 10^7 formalized isogenic leukaemic cells, the injection being repeated every 4 days for 16 days; group d) comprised 15 mice which received the combination of both treatments.

2. *Leukaemia E ♀ K1.* 75 C_{57}Bl/6♀ mice, aged 2 months, received by intravenous route 10^3 cells from E ♀ K1

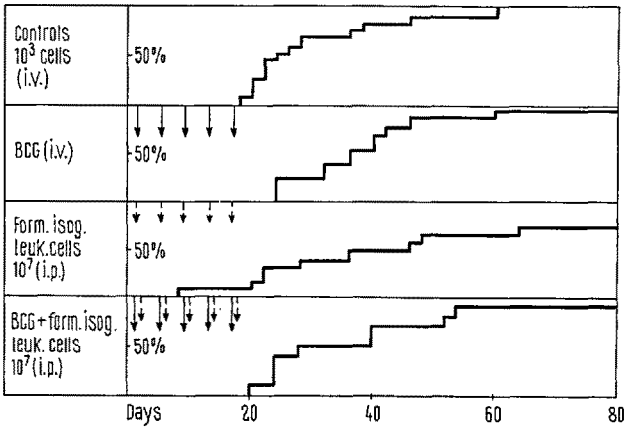

Fig. 1. Cumulative survival of mice carrying E ♀ K1 leukaemia not treated, or treated by B.C.G., or formolinized isogenic leukaemic cells, or by combination of both.

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