The Dose Reduction Factor (DRF) at the $LD_{50/60}$ level was 1.65, demonstrating that 5-HT facilitated the planarians' recovery from X-rays.

Many radioprotectors have been described in the literature, but only a few substances applied after irradiation decrease the effects induced by radiation. NERURKAR et al.[3] and SAHASRABUDHE[4] showed that methionine applied after irradiation is effective in recovery of nucleic acid synthesis, but BACQ and BEAUMARIAGE[5] considered it insufficiently important to affect mortality. KANAZIR et al.[6] and BEĆAREVIĆ et al.[7] found that X-irradiated rats treated with nucleic acids survived lethal doses of radiation. BEĆAREVIĆ and his collaborators interpreted this recovery effect as a depression of the cellular metabolic rates of the irradiated rats or as a result of the inclusion of biologically active nucleic acids which would permit the recovery of metabolic processes.

Percent of mortality in planarians at different dose of X-rays with and without posttreatment of serotonin-creatinine sulfate complex

Exposure in Roentgens (R)	Mortality (%)	
	Control	Post-treated
300	0	0
400	5	0
500	10	0
600	25	2
700	60	2
800	72	5
900	82	22
1000	92	22
1100	95	42
1200	97	52
1300	97	77
1400	100	82
1500	100	85
1600	100	90
1700	100	95
1800	100	100
2000	100	100

SUGAHARA et al.[8] found that precursors of nucleic acids increase the survival time of mice receiving repeated sublethal doses, but the recovery effect seemed to be limited to sublethal damage.

When dormice (*Glis glis*) were exposed to lethal dose of X-rays during hibernation and cysteine was injected when the animals were brought 3 weeks after irradiation to room temperature, no mortality occurred within the following 30 days[9]. Cysteine and cysteamine applied after irradiation to swell the seeds of *Vicia faba*, reduced the mitotic inhibition[9].

In our experiments 5-HT applied after irradiation in planarians demonstrated its efficacy in restoration of the recovery mechanism altered by X-rays.

Resumen. En este trabajo, se demuestra que el complejo de serotonina-sulfato de creatinina (5-HT) no solamente es un radioprotector efectivo de las planarias contra los rayos X, sino que también interviene en la restauración de los mecanismos de récuperación cuando se aplica como postratamiento a la irradiación.

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Growth Inhibitory Effects of Adenosine 3′,5′-Monophosphate on Mouse Leukemia L-5178-Y-R Cells in Culture

The importance of adenosine 3′,5′-monophosphate (cyclic AMP) in normal cell functions and metabolism is becoming increasingly apparent in many tissues and organs[1,2] and it has been recently reported to inhibit the growth of transplanted NKL-lymphosarcoma in mice and the multiplication, as measured by viable counts, of tumorigenic cell lines in vitro[3–5]. However, the mechanism by which cyclic AMP inhibits the cancer cells is at present unknown.

During our study of the inhibitory action of heat-inactivated antisera, in the absence of complement, on mouse leukemia L-5178-Y-R cells in culture, we found cause to speculate that some indirect mechanisms triggered as a result of antibody-antigen reactions on the cell membrane might be involved[6]. Growth inhibitory effects of the antisera are of a rather slow process, as are those of hormonal effects on the cells, starting at 4 to 6 h after the experiment, with maximal effects at 24 to 48 h. Systems around pyruvic acid cycle seem to be involved. Glucose, succinate, nicotinamide, but not malate, are able to alleviate, at least partially, the harmful effects of antisera. These phenomena are very reminiscent of the second messenger effect found in hormonal systems. Furthermore, activation of adenyl cyclase in the sea urchin egg membranes has been reported at fertilization[7]. We, therefore, investigated whether cyclic AMP would mimic the effects of antisera on L-5178-Y-R cells. The results are remarkably similar.

We now report the effects of cyclic AMP on the mouse leukemia L-5178-Y-R cells in culture. The experimental method used was the combination of the previously reported[6] and that of the modified BOLLUM's[8,9]. Two

sets of triplicate cultures with an initial inoculum of 1×10^5 (for viable count study) or 2.5×10^5 (for both viable count and radio-isotope labelling study) viable cells were set up in Bellco tissue culture tubes in 5 ml amounts. Adenosine 3′, 5′-monophosphate (cyclic AMP, Calbiochem) and N^6-2′-*O*-dibutyryl-adenosine 3′, 5′-monophosphate (dibutyryl cyclic AMP, Boehringer-Mannheim) were dissolved in warm distilled water to make stock solutions of 10 mg/ml and filtered through a prewashed Millipore filter. Similar volumes of filtered distilled water were used as controls. For dose-response study, 0.2 ml of the two-fold dilutions of the stock solutions were added to the cultures at the start of the experiments to make final concentrations of 400 µg, 200 µg, 100 µg, 50 µg, 25 µg and 12.5 µg/ml of culture. At 24 and 48 h, viable counts were made. For time-course study, final concentration of 200 µg/ml (0.40 m*M* for dibutyryl cyclic AMP and 0.53 m*M* for cyclic AMP) was used.

At 0, 1, 2, 4, 6, 8, 24 and 48 h after the start of experiment, each set of triplicate cultures were pooled, mixed, re-distributed equally into 3 tubes and ^{3}H-thymidine, ^{3}H-uridine, and ^{14}C-2-glycine (New England Nuclear Corp.) were added to make final concentrations of 1 µc, 4 µc and 1 µc/ml respectively. They were pulse labelled for 60 min in 37 °C water bath, stopped by chilling, viable cell counts were made and processed by conventional TCA extraction methods[6] and modified Bollum's filter paper method[8,9], finally assayed in Packard Tri-Carb liquid scintillation spectrometer. Experiments were repeated 2–4 times.

The Table shows the results of dose-response study. Dibutyryl cyclic AMP inhibited the cell growth, even at the lowest concentration, i.e., 12.5 µg/ml (0.025 m*M*) used, while cyclic AMP was much less effective and inhibited the cells only at the concentration of 200 µg/ml (0.53 m*M*) or higher. At the concentrations of 50 to 25 µg/ml (0.133–0.066 m*M*), cyclic AMP showed some stimulatory effects. These are in accord with the known facts that dibutyryl cyclic AMP penetrates the cells better and is more resistant to the action of specific

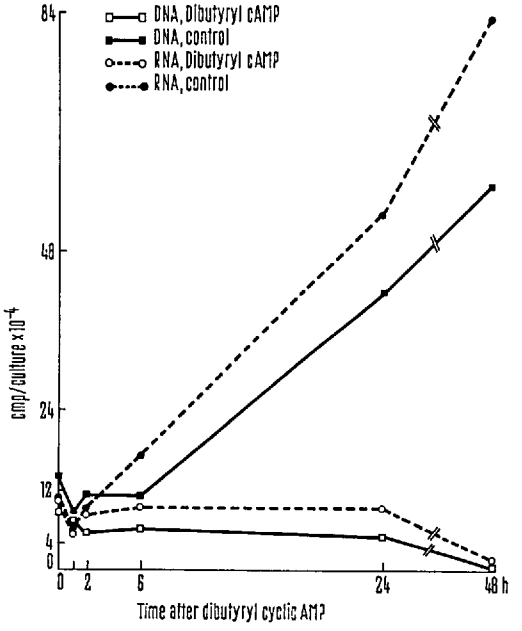

Fig. 1. Effects of dibutyryl cyclic AMP on the rates DNA and RNA synthesis in cultures of L-5178-Y-R cells. Cell inoculum at 0 h, 2.5×10^5 viable cells/ml; dibutyryl cyclic AMP conc., 200 µg/ml (0.4 m*M*). Labelling time 60 min.

Comparison of inhibition of L-5178-Y-R cells by dibutyryl cyclic AMP and cyclic AMP. Percent inhibition = [viable cell count (control) — viable cell count (cyclic AMP) ÷ viable cell count (control)] × 100

Compound	Concentration (µg/ml)	Concentration (m*M*)	Inhibition (%) 24 h	Inhibition (%) 48 h
Dibutyryl cyclic AMP	400	0.800	59	94
	200	0.400	60	85
	100	0.200	67	71
Cyclic AMP	400	1.060	43	73
	200	0.530	—10	22
	100	0.265	8	1

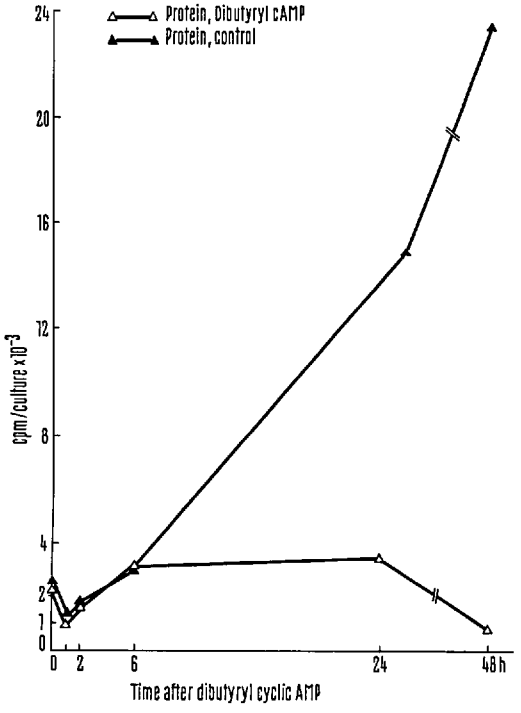

Fig. 2. Effects of dibutyryl cyclic AMP on the rate of protein synthesis in cultures of L-5178-Y-R cell. Experimental conditions same as in Figure 1.

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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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