Bauer, G., R. R. Friis, H. Mattersberger, and P. H. Hofschneider. 1978. Controlled release of particle-associated RNA-dependent DNA polymerase by primary chick embryo cell cultures. Exp. Cell Res. 117, 383 - 392.

Bauer, G. and H. M. Temin. 1979. RNA-directed DNA polymerase from particles released by normal goose cells. J. Virol. 29, 1006 - 1013.

Bauer, G. and H. M. Temin. 1980. Radioimmunological comparison of the DNA polymerases of avian retroviruses. J. Vir. 33, 1046-1057.

Bauer, G. and H. M. Temin 1980. Specific antigenic relationships between the RNA-dependent DNA polymerases of avian reticuloendotheliosis viruses and mammalian type C retroviruses. J. Virol., April issue.

SHORT CHAIN RNA FRAGMENTS AS PROMOTERS OF LEUKOCYTE AND PLATELET GENESIS IN ANIMALS DEPLETED BY ANTI-CANCER DRUGS

Mirko Beljanski, Michel Plawecki, Pierre Bourgarel and Monique Beljanski
Laboratoire de Pharmacodynamie, Faculté de Pharmacie, 92290 Châtenay-Malabry, France

SUMMARY

We describe the biological activity of single-stranded short-chain RNA fragments obtained in large amounts by mild degradation of purified Escherichia coli ribosomal RNA with pancreatic RNase A . In vitro , these RNA fragments , termed $BLR_{(s)}$, can be used by DNA dependent DNA polymerase I as primers to initiate the replication of DNA isolated from rabbit bone marrow and spleen . They concentrate in these tissues when given i.v. or per os to rabbits and can restore normal leukocyte and platelet counts after these have been dangerously decreased by various antimitotic drugs. These actions are manifest within 24 h for leukocytes and 72 h for platelets . The RNA fragments are devoid of toxicity for animals and do not induce tolerance phenomenon . When polynuclear/lymphocyte ratio is upset by cyclophosphamide , the RNA fragments induce white blood cell differentiation and a differential increase of polynuclear and lymphocyte counts until their normal ratio and levels are fully restored . The RNA fragments do not act as primers for the in vitro replication of cancer DNA and do not stimulate tumor cell multiplication in mice . They appear to act on physiologically normal cells involved in leukopoiesis and platelet formation .

ABBREVIATIONS

RNA , ribonucleic acid ; DNA , deoxyribonucleic acid : Leuco-4 (adenine + phosphate) ; RNase , ribonuclease ; $BLR_{(s)}$, Beljanski leukocyte restorers ; DEDTC , diethyldithiocarbamate.

INTRODUCTION

It is well known that the primary step of cell division requires the replication of DNA by DNA dependent DNA polymerase which , in different biological systems , uses special naturally produced short chain RNA as primers to initiate a new DNA chain and thus make cell multiplication possible (1)(2)(3)(4)(5)(6)(7).

The transfer of genetic informations mediated by purified RNA in different biological systems has been reported by several authors (8) (9) (10)(11)(12)(13)(14). In higher organisms the development of specific tissue in response to exogenous RNA during organ culture has been described (15) (16) (17) (18). Various examples show that RNA plays a part in cell regulation . For instance , according to their nature and their affinity for a given DNA , RNA may , depending on the prevailing physiological conditions , force a cell to lose or to recover the ability to follow the normal regulation process . Thus , purine rich small size RNA (6 S) and RNA fragments (25 - 50 nucleotides) prepared by degradation of r- RNA with ribonuclease A may act on gene expression in plants (19) (5) and animals (20) (21) . When inoculated into young axenically cultured Datura stramonium (19) or inverted stem sections (5) , these short-chain RNA initiate the appearance of transplantable tumors , but only if the solid culture medium contains large amounts of auxine . Other RNA fragments , prepared using a different nuclease , can arrest tumor cell development in plants (22) . It has also been reported that special small molecular weight RNA can achieve specific tumor regression in animals (23) (24). Purine-rich RNA fragments can prevent DNA virus proliferation in rabbits (20).

In previous articles , we showed that specific short chain RNA fragments obtained by mild degradation of bacterial r-RNA with

ribonuclease A can act as primers for in vitro replication of DNA. These primers are single stranded RNA fragments and their nucleotide compositions determine their specificity of action : some act as primers for the replication of viral DNA (20) and others for the replication of plant (5) , bacterial or mammalian DNA (7) (2) . Primer RNA are not transcribed into DNA but simply initiate the DNA replication process ; they are rapidly eliminated once the replication is in progress. We expected their priming action to involve an effect on gene regulation. It seemed possible to gain specific harmless control over the multiplication of traget cells by acting solely on the replication and expression of the genetic material without altering genes and cell structure. This is a very important point , because , in most cases , agents which inhibit cell multiplication have harmful side effects : for instance , antimitotic drugs used for cancer treatment have an inhibiting effect on leukocyte and blood platelet stem cells or their derivatives . We expected that , under appropriate conditions , those RNA fragments which selectively prime spleen and bone marrow DNA in vitro replication might be able to stimulate stem cell division (through DNA replication) , with the consequent restoration of normal amounts of circulating leukocytes and platelets in depleted animals.

In the present report , we describe the properties of these RNA fragments (named $BLR_{(s)}$) which exert a remarkable effect on leukocyte and blood platelet formation in laboratory animals.

BLR - RNA fragments may be obtained in large amounts by pancreatic ribonuclease digestion of purified Escherichia coli ribosomal RNA. These fragments have been fully characterized both physically and chemically and their in vitro and in vivo activities are described below.

MATERIALS and METHODS.=

Chemicals and biological products : Phenol (grade A) and chloroform (reagent grade) were obtained from Prolabo, Paris , France and sodium lauryl sulfate from Serlabo, Paris,France. Pancreatic RNase (4 x crystallized) and deoxyribonuclease (grade A) were supplied by Worthington , Freehold, N.J. USA ; poly AG , Poly I-Poly C and (^{3}H)-Poly A (s.act.94 µci/mole P , 3 %) by Miles Laboratories , Elkhart, Ind. USA ; Endoxan (cyclophosphamide) by Laboratories Lucien , Colombes, France; methotrexate (amethopterin) by Specia,Paris, France , and Leuco-4 (adenine + phosphate) by Leboratoire H.Villette,Paris France ; Daunorubicin (anthracyclin) was gift of Dr. R.Marsal, Rhône-Poulenc, Ivry/Seine,France ; sodium-diethyldithiocarbamate and Endotoxin E.coli 026 B6 Difco were gifts of Dr. Roumiantzeff, Institut Mérieux,Lyon,France. (^{3}H)-uracil (44,5 ci/mmol) and (^{3}H)-guanine (7,5 ci/mmol) were obtained from the Commissariat à l'Energie Atomique, Saclay,France. Rabbits (3-4 kg) were white "Bouska", New Zealand. Mice were Swiss.

Bacteria and growth conditions. Escherichia coli T 3000 (K 12) a non pathogenic strain was used . Cells were grown aerobically in synthetic medium at 36°C (25) and harvested during the exponential phase of growth. When radioactive RNA were required cells were labelled for 6 h during exponential growth by addition of 500 µci (^{3}H)-guanine and 500 µci (^{3}H)-uracil per liter of synthetic medium .

Isolation of ribosomes and ribosomal RNA . Ribosomes were prepared from cells as previously described (26) and deproteinized by treatment with phenol and chloroform . Ribosomal RNA were dissolved in 0.01 M Na acetate, 0.1 M NaCl pH 5.0 and the solution was centrifuged for 30 min. at 40,000 g to remove most polysaccharide contaminants (27). The supernatant solution was dialysed against 0.1 M KCl for 16 h at 4°C and then against distilled water for 1 h at 4°. The yield of RNA was determined by measurement of A 260 nm and integrity of r-RNA was checked by polyacrylamide gel electrophoresis (28) (23 S and 16 S = 1.6 x 10^6 and 0.5 x 10^6 daltons)

Degradation of ribosomal RNA by pancreatic RNase A. Pancreatic RNase A (15 mg) was added to a solution of r-RNA (1 g) in distilled water(50 ml) and the mixture was incubated at 36°C for 20 min. Reaction was stopped by adding 1 volume of phenol containing 10% (v/v) distilled water, the mixture was stirred at room temperature for 10 min. and centrifuged at 12,000 g for 10 min. to separate phenolic and aqueous phases . The aqueous

phase was removed and treated again as before with 1 volume of 10 % aqueous phenol . The second aqueous phase was then shaken for 10 min. with 1 volume of chloroform, and after centrifugation the aqueous phase was recovered . This step was repeated three times and the final aqueous phase was dialysed against 2.5 liter of distilled water for 16 h at 4°C. under axenic conditions. The amount of non dialysable RNA was determined by measurement of A 260 nm (yield 50-60 %); the dialysed solution was lyophilized and the residue was stored in a dry environment . The product which stimulates leukopoiesis and platelet formation are termed $BLR_{(s)}$ (see abbreviations). $BLR_{(s)}$ were characterized by determination of electrophoretic mobility in polyacrylamide gels and U.V. absorption spectra .

Analytical tehniques. Proteins were measured by Lowry method (29) and DNA by the diphenylamine reaction (30). RNA base ratios were determined as follows : 150 µg of $BLR_{(s)}$ were hydrolysed at 100°C in N HCl for 1 h (boiling water bath), the hydrolysate was evaporated to dryness in a dessicator and the residue was dissolved in 0.05 ml of distilled water. Samples of the solutions were analysed by chromatography on Ecteola cellulose plates according to Björk and Svensson (31). UV absorbing spots were located (purine bases and pyrimidine nucleotides) cut out and eluted with 0.1 N HCl . The concentrations of eluted compounds were determined using the following coefficients absorption maxima : A, 13 ; G , 12,8 ; C = 11 ; U, 10 (32).

Determination of leukocyte and platelet count in circulating blood of rabbits.

Blood taken from rabbit marginal veins was mixed immediately with EDTA and leukocyte and platelet counts were determined using a Coulter counter model F. Differential leukocyte counts were made using the May-Grünwald Giemsa staining method and 200 - 300 leukocytes were counted under the microscope by the same observer . Leukocytes were classified as polynuclears (including neutrophil, eosinophil and basophil cells) and lymphocytes (including monocytes).

Isolation of DNA and DNA polymerase. DNA was isolated from gently broken tissue by phenol/chloroform extraction in the presence of 2 M $NaClO_4$ and 0.01 M EDTA (33).

E.coli DNA dependent DNA polymerase I (EC 2.7.7.7.) was partially purified from bacterial extracts as previously described (34). This preparation contains some phosphatase activity for removing phosphate from 3'-ribonucleotides.

R E S U L T S

Biochemical properties of $BLR_{(s)}$. Chemical and physical characterization of

$BLR_{(s)}$ shows that they are single stranded RNA fragments (no hyperchromicity) almost devoid of DNA and protein contaminants(0.3 and 0.5 % respectively). Base ratio analysis shows that they contain an excess of purine over pyrimidine bases (G+A/C/U = 2.4 while in intact r-RNA this ratio is close to 1.12, Table 1). This is not surpirsing since RNase A cleaves RNA chains solely at C and U residues. The average electrophoretic mobility of $BLR_{(s)}$ is the same as that of poly A preparation which corresponds to m.w. of 1.7×10^6 daltons (Fig.1). This suggests that these RNA fragments contain on an average about 50 nucleotides which is within the size range of DNA replication primers . Their priming action on DNA replication in vitro was tested using bone marrow and spleen $DNA_{(s)}$. The choice of these $DNA_{(s)}$ was guided first by the observation that injected radioactive $BLR_{(s)}$ are found in relatively high amount in the bone marrow and spleen , and second by the need to ascertain wether $BLR_{(s)}$ act differently on DNA of normal and cancerous cells . When DNA polymerase I is incubated in a complete system containing a radioactive deoxyribonucleoside-5'-triphosphate but no added RNA primer it is able to synthesize a very limited amount of DNA in a reaction which quickly reaches a plateau. This synthesis is probably due to the fact that the enzyme used here is only partly purified (when highly purified it functions less well) and contains some material of primer RNA type . When $BLR_{(s)}$ are added to such a system using bone marrow (Fig. 2) or spleen DNA (results not shown) as template DNA replication is highly stimulated whereas when brain (Fig. 3) or kidney DNA (results not shown) is used as template no stimulation is seen . These results indicate that $BLR_{(s)}$ may be potential in vivo DNA replication primers in the bone marrow and spleen where are retained. We next asked whether $BLR_{(s)}$ stimulate DNA replication in the same way in healthy and cancerous tissue. When DNA from healthy breast and lung tissue was used as template in a complete system a slight stimulation of replication was observed. On the contrary when template DNA was prepared from cancerous tissue , replication was slightly slowed down by the presence of $BLR_{(s)}$ (Fig.4). These results indicate, but do not constitute proof, that $BLR_{(s)}$ will not stimulate DNA replication in vivo and multiplication of malignant cells .

In vivo localization of radioactive $BLR_{(s)}$ in the rabbit. Preliminary experiments in mice had shown that , after i.v. injection , (^3H)-$BLR_{(s)}$ were mostly found in the spleen and in the bone marrow ; radioactivity slowly decreased and could not be detected for more than 2 to 3 weeks. When the distribution of radioactive $BLR_{(s)}$ in the rabbit was determined the results shown in Fig. 5 were obtained . The fact that high specific activity is

TABLE 1

Base ratio (G+A/C+U) of r-RNA and $BLR_{(s)}$ obtained by degradation of the same r-R

Bases	Moles per 100 moles of analysed nucleotides		
	r-RNA (23 S + 16 S)	$BLR_{(s)}$ obtained with pancreatic RNase A	
		Sample 1	Sample 2
G	28.0	42.0	40.5
A	24.9	28.8	29.8
C	24.1	15.7	16.5
U	23.0	13.5	14.2
G+A/C+U	1.12	2.42	2.36

$BLR_{(s)}$ and r-RNA were hydrolysed and analysed as described in Material and Meth

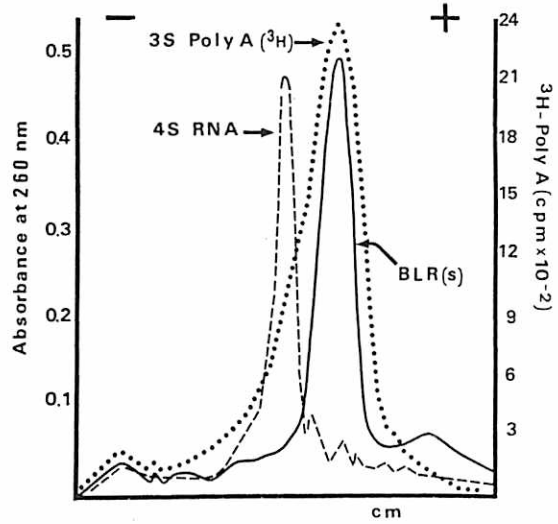

Fig.1 Polyacrylamide gel electrophoresis of $BLR_{(s)}$. $BLR_{(s)}$ (4 µg) were mixed with (^3H)-Poly A (3 S) (containing 14,000 CPM) used as a marker and submitted to electrophoresis on polyacrylamide gel (5 % (W/v) for 90 min. at 5 mA per tube at 4°C and the position of U.V. absorbing material for $BLR_{(s)}$ was detected as previously described (9). The position of (^3H)-Poly A was determined by measuring the radioactivity in very thin slices of polyacrylamide gel using a Packard liquid spectrometer (Prias). 4 S RNA was separately run as a marker and densitometer tracings are superposed on this Fig.

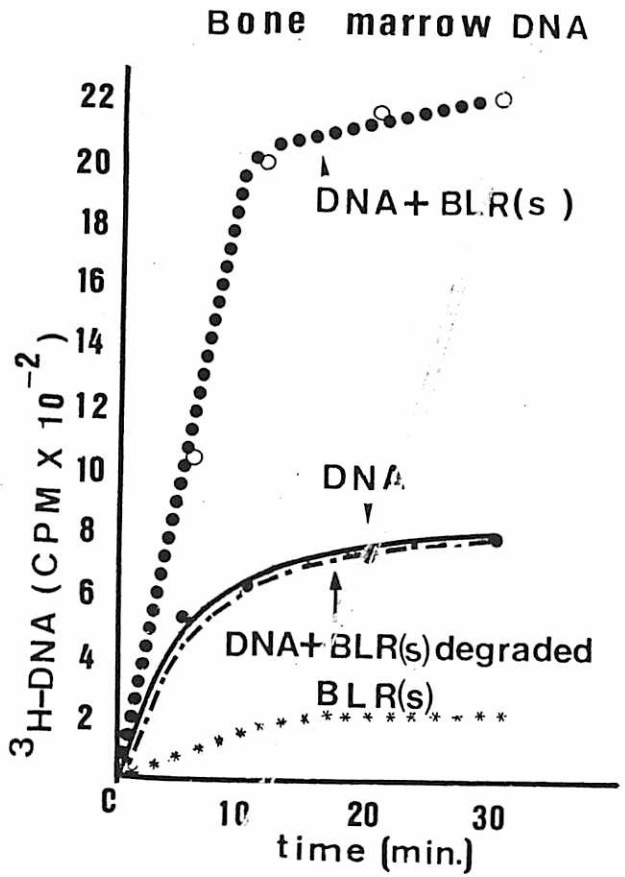

Fig.2 $BLR_{(s)}$ act in vitro as primers for bone marrow DNA replication. Incubation mixture contained per 0.15 ml : Tris-HCl buffer pH 7.65 , 25 µMoles; $MgCl_2$: 2 µMoles ; four d-XTP : each 5 nanomoles (+ (^{3}H)-TTP , 50,000 CPM) ; DNA : 0.2 µg ; $BLR_{(s)}$: 4 µg ; DNA dependent DNA polymerase I, 80 µg . Incubation : 10 , 20 and 30 min. at 36°C. TCA (trichloroacetic acid)-precipitable material was filtered on GF/C glass filter, washed, dried and radioactivity measured with a Packard liquid spectrometer (Prias). $BLR_{(s)}$ were degraded by incubation in 0.3 N KOH at 36°C for 16 h , then neutralized. Analysis were carried out in triplicate for each incubation time .

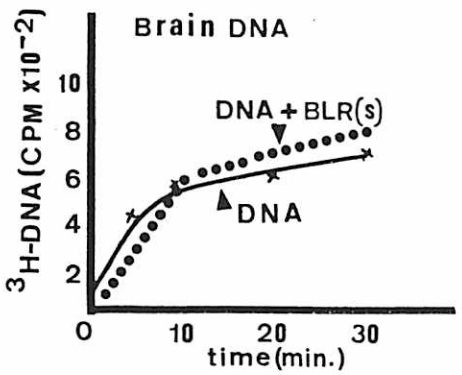

Fig.3 $BLR_{(s)}$ do not prime for monkey brain DNA replication ; see conditions described in the legend to Fig. 2

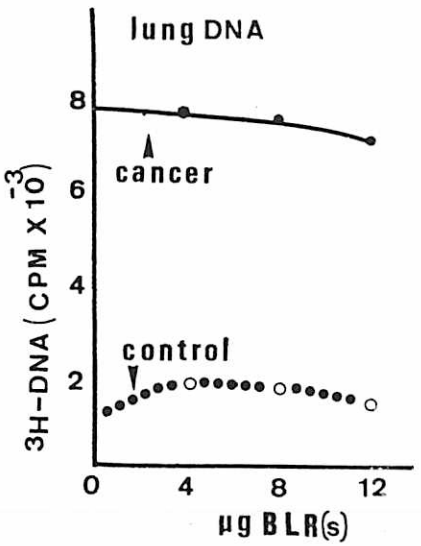

Fig.4 $BLR_{(s)}$ and in vitro replication of DNA from human lung , healthy and cancerous tissues . For incubation conditions , see legend to Fig.2. BLR concentrations : 4 , 8 and 12 µg (in duplicate). Incubation time 10 min. at 36°C .

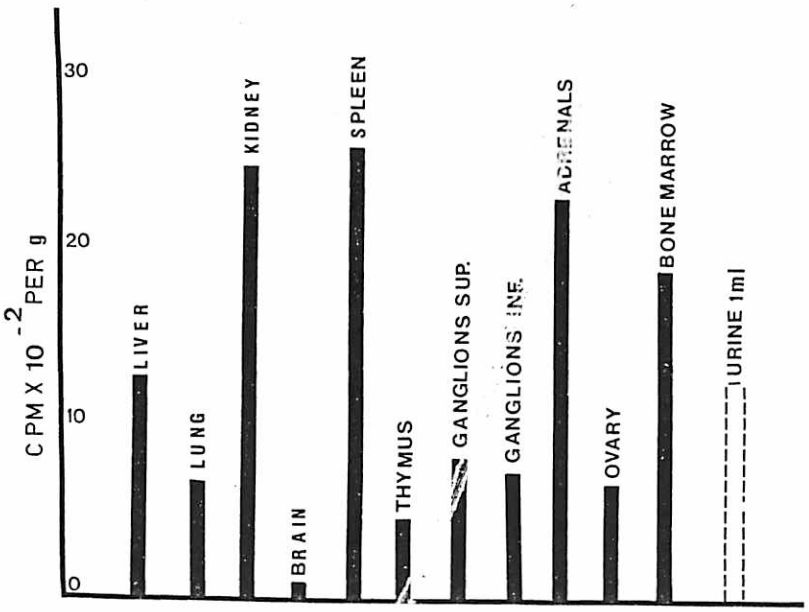

Fig.5 In vivo localization of radioactive $BLR_{(s)}$ in the rabbit . 5 mg of (^{3}H)-$BLR_{(s)}$ (3 x 10^6 CPM) (labelled with (^{3}H)-guanine and (^{3}H)-uracil) are injected into a 3 kg normal rabbit . 24 h later , the animal is anesthesized and sacrificed . Samples of 0.2 to 0.5 g of organs are analysed for hot trichloroacetic acid soluble radioactivity (5 % final conc. of TCA , 100°C , 30 min). The results (mean values for three rabbits) are expressed as CPM / g of wet material . Urine was collected from the bladder for radioactivity measurement .

found in the femur bone marrow and spleen suggested that $BLR_{(s)}$ might act on DNA replication in the cells which give rise to leukocytes and platelets. The presence of radioactive material in adrenal glands, kidney and liver may reflect blood flow or possibly reutilization of radioactive nucleotides by organs which synthesize $RNA_{(s)}$. The excretion of degraded radioactive material found in urine 24 h after (^{3}H)-BLR injection show that $BLR_{(s)}$ are biodegradable (Fig.5). However , in the adrenal glands, which contain stress stimulated haemetopoïetic cells (35), $BLR_{(s)}$ could exert a stimulating action.

Degradation of $BLR_{(s)}$ by rabbit plasma. In order to get some information about the extent of BLR degradation , plasma of circulating blood from healthy rabbits was prepared and incubated with (^{3}H)-$BLR_{(s)}$ at 36°C in function of time. Parallel experiments were performed with (^{3}H)-Poly C or Poly A. Alcohol precipitable material was filtered on millipore, dried and its radioactivity measured. The half life of (^{3}H)-$BLR_{(s)}$ was 8 min. and that of Poly C , 1 min., while Poly A was poorly degraded (Fig.6). $BLR_{(s)}$ which contain an excess of purine bases show a relatively important resistance to plasma RNase action. The activity of plasma RNase was also tested after rabbits had received $BLR_{(s)}$ i.v. Blood plasma was prepared just before injection and every h thereafter for 6-7 h. Fig.7 shows that after BLR injection, plasma RNase exhibit higher degradation activity toward (^{3}H)-BLR than that found in the plasma before BLR administration. Increased RNase activity is manifest during 5 - 6 h and then returns to normal value. These observations do not tell us if injected $BLR_{(s)}$ induced the increase of the amount of plasma RNase, or if some RNase activating factor (s) appeared in the presence of $BLR_{(s)}$. The period of increased plasma RNase activity corresponds to fever response induced by BLR injection to rabbits (Fig. 15).

$BLR_{(s)}$ activity and integrity. Hydrolysis of $BLR_{(s)}$ to nucleotides by incubation of 16 h in 0.3 N KOH at 36°C completely abolishes their stimulating activity in vitro (Fig.2) and in vivo (Fig.9). Treatment with RNase A for 2 h at 36° followed by deproteinisation and dialysis of the incubation mixture also reduces the activity of $BLR_{(s)}$ but does not completely abolish it.

Excellent priming activity of $BLR_{(s)}$ in DNA replication might be explained by the presence , in DNA dependent DNA polymerase as well as in RNase A preparations even crystallized several times, of phosphatase activity(verified for both types of enzymes). Phosphatase removes 3' phosphate giving rise to 3'OH group necessary for priming activity. The endogenous enzyme does it in animals. We have controlled that $BLR_{(s)}$ retreated with E.coli alkaline phosphatase exhibit normal leukopoietic activity.

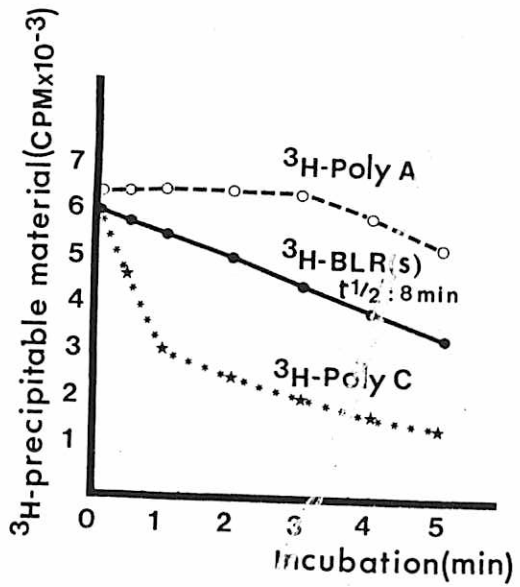

Fig. 6 In vitro degradation of (^{3}H)-BLR by blood plasma of healthy rabbits. For each time indicated on the Fig , incubation mixture (final volume 0.15 ml) contains 25 µMoles of Tris-HCl buffer pH 7.65 ; 200 µg of (^{3}H)-BLR$_{(s)}$(7000 CPM) ; 0.01 ml of plasma (0.7 mg of protein). A parallel experiment was performed with (^{3}H)-Poly A (200 µg ,7.200 CPM) and (^{3}H)-Poly C (200 µg, 7,160 CPM) . Incubation at 36°C. ; time is indicated on the figure . To stop the reaction , alcohol (95°) was added in excess and 5 µMoles of KCl to precipitate the (^{3}H) labelled insoluble material . The precipitate was filtered on glass GF/C millipore filter , washed with alcohol , dried and its radioactivity was measured with Packard liquid scintillation counter (Prias). Results are expressed as CPM . Half life (t 1/2 = 0.693/K) was calculated using the values in the linear portion of the curves.

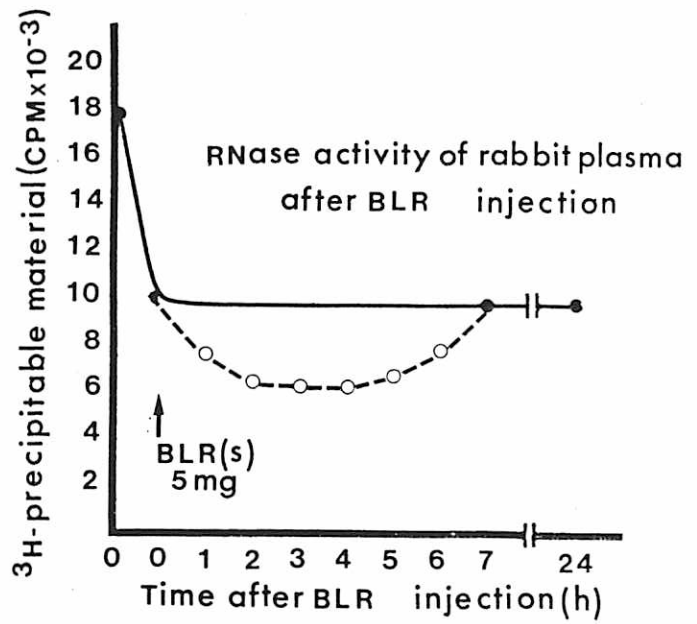

Fig.7 Increase of RNase activity in plasma of rabbits injected with BLR$_{(s)}$. For incubation conditions , see legend to figure 6 . 200 ug of (^{3}H)-BLR$_{(s)}$(18,000 CPM) were used for degradation with rabbit plasma (RNase) which was prepared from blood taken before BLR i.v. injection and every h thereafter for 6 - 7 h .

Leukopoietic activity of $BLR_{(s)}$ in rabbits permanently treated with Endoxan. Endoxan is a commercial preparation containing 75 % cyclophosphamide, an alkylating agent which can form alcoyl bridges between the strands of DNA and thus prevent its transcription into RNA. It is used for experimental and human cancer therapy and it is believed that in the body cyclophosphamide is converted into an active substance and non cytotoxic product (36). When used for human cancer therapy a total weekly dosage of 300 mg Endoxan is commonly prescribed. This is equivalent to about 0.75 mg/kg/day. Our rabbits received per kg i.v. dosages about 40 times higher , which for many animals prove lethal within 8 to 12 days.

I.V. administration of $BLR_{(s)}$. White rabbits (3-4 kg) were given 100 mg Endoxan i.v. daily , and blood samples were taken at daily intervals and leukocytes, erythrocytes and platelets counted. When necessary, polynuclears and lymphocytes were counted separately. When leukocyte count had fallen from 7,000 - 12,000 (healthy rabbit) to 3,000 - 5,000/mm^3 blood, rabbits received i.v. 1-6 mg $BLR_{(s)}$ in sterile physiological saline solution. All $BLR_{(s)}$ samples were active in increasing the leukocyte count in Endoxan treated rabbits and as shown in Fig.8 could not be replaced for leukocyte restoration by E.coli endotoxin. Their effect was proportional to the amount injected until a saturation dosage was reached after which no further increase occured (Fig.9). A normal leukocyte count was usually restored by i.v. injection of 2.5 mg of $BLR_{(s)}$, and significant leukopoïetic activity was obtained with 0.5 mg $BLR_{(s)}$ / 100 mg Endoxan / 3.5 kg rabbit. However individual variations among rabbits are such that the $BLR_{(s)}$ dosage may have to be doubled to obtain the same response. The increased leukocyte count obtained with $BLR_{(s)}$ reaches a normal value 24-48 h after their injection , remains at a high level for a few days and then returns to the value found before injection of $BLR_{(s)}$. The duration of cycle of $BLR_{(s)}$ induced changes in leukocyte count is about 7 days (Fig.10). Increased leukocytes obtained with $BLR_{(s)}$ never exceeded 250 % of the lowered count produced by Endoxan treated nor 100 % of the normal value in untreated rabbits. These results suggest that a very powerful cellular regulation process is involved. This process is not perturbed by excess dosages of $BLR_{(s)}$ since in Endoxan treated rabbits given 1-6 mg $BLR_{(s)}$ i.v. evry second day there is no additional increase in leukocyte counts as the dose is increased. In addition there is no decrease in the response such as might have been caused by a possible mass action of repeated doses, a possible toxic effect or the development of resistance to $BLR_{(s)}$. A fresh dose of $BLR_{(s)}$ is observed to stimulate leukopoiesis only when the preceeding dose has ceased to act (Fig.10). This numerous repeated doses of $BLR_{(s)}$ do not have a cumulative effect of any kind and do not lead to a loss of leukopoietic stimulating effect.

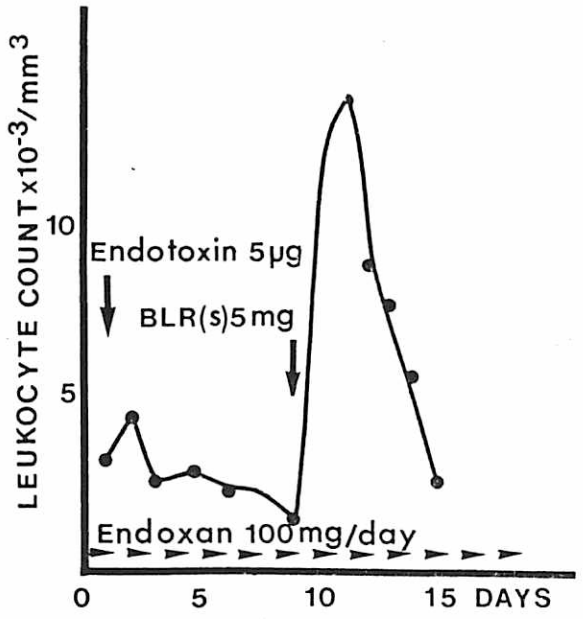

Fig.8 Effect of E.coli Endotoxin and $BLR_{(s)}$ on the leukocyte count in the Endoxan-treated rabbit . E. coli Endotoxin (5 µg) was given i.v. to 3 kg rabbit which have been treated for 7 days with 100 mg Endoxan/ day . No appreciable effect on leukopoïesis was noted. As shown by the arrow when these same rabbits received 5 mg $BLR_{(s)}$ i.v. the leukocyte count increased . The mean increase of leukocyte count (6 rabbits) after endotoxin i.v. injection was 14 % $\pm$ 3 % , $p < 0.02$.

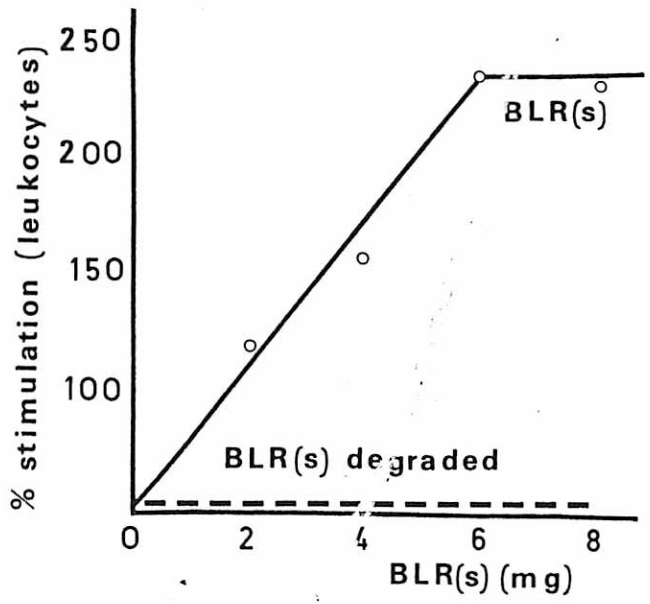

Fig.9 Increase in leukocyte count in Endoxan treated rabbits given various amounts of $BLR_{(s)}$. 3.5 kg rabbits were treated with Endoxan (100 mg injected i.v.) daily for four weeks . The first dose of $BLR_{(s)}$ (0.5 mg) was injected i.v. on day 1, 10 min after Endoxan injection ; when the stimulating effect of the first dose ended , a second 1 mg dose was given i.v. etc... Circulating leukocytes were counted with a "Coulter counter ". The same results were obtained with three other rabbits. $BLR_{(s)}$ were degraded by incubation in 0.3 N KOH at 36°C for 16 h , then neutralized.

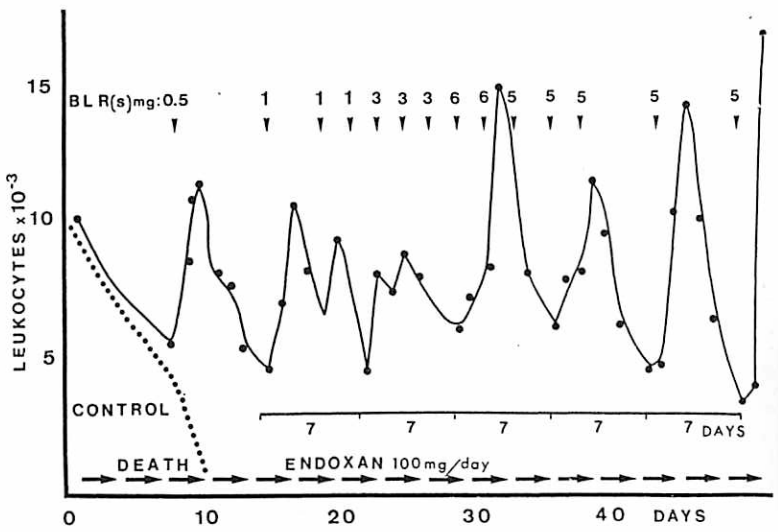

Fig. 10 Leukocyte count in the Endoxan treated rabbits receiving varying doses of $BLR_{(s)}$ every second day . After leukocyte count had been strongly decreased , a 3.5 kg Endoxan treated rabbit (100 mg/day) received varying doses of $BLR_{(s)}$ ranging from 1 to 6 mg every second day as shown by the arrows . Circulating leukocytes were counted daily with a Coulter Counter . The results given in this Fig. are an average obtained with 10 rabbits . The mean increase in leukocyte count was 172 % $\pm$ 17 % as standard error . The confidence interval calculated using paired sample student's t test $p < 0.001$.

Although Endoxan dosage at the level used in these experiments proves lethal in many sensitive animals after 8-12 days this effect is suppressed by simultaneous treatment with $BLR_{(s)}$. In one experiment five rabbits which received 100 mg Endoxan daily and 5 mg $BLR_{(s)}$ at regular intervals for a period of two and one half months were all alive in good condition 2 - 3 years after cessation of the treatment. $BLR_{(s)}$ procured survival of 94 rabbits (over 100 used) which received Endoxan for 30-90 days . Six died as a result of bacterial infection combined with high sensitivity to Endoxan. In the 94 survivors the mean increase in leukocyte count was 154 % , standard error $\pm$ 11.% and $p < 0.001$ by standard Student's t test.

As far as erythrocyte genesis is concerned, preliminary results show that in rabbits $BLR_{(s)}$ are active in this process only under particular conditions (unpublished results).

Effect of $BLR_{(s)}$ in control rabbits receiving no drugs. After i.v. administration of 5 mg of $BLR_{(s)}$ to healthy rabbits leukocyte count increases from 8,000 to 14,000 in 24 h and returned to its original value one day later. Higher doses are controlled in other rabbits. For example after i.v. injection of 50 mg of $BLR_{(s)}$ the leukocyte count in a healthy rabbit rose from 7,300 to a maximum of 17,500 in 20 h and returned to its original value two days later.

After injection of $BLR_{(s)}$ a short transcient fall in cell count is observed before stimulation occurs. This is seen after a four h delay for leukocytes, and a 24 h delay for platelets. For example in the experiment mentioned above in which a rabbit received a single 50 mg dose the leukocyte count had fallen from 7,300 to 3,300 four h after injection but was 17,500 twenty h later. This reaction is apparently independent of the amount of $BLR_{(s)}$ administered (range tested : 3 - 50 mg per rabbit weighing 3-4 kg). Furthermore the same initial fall in leukocyte and platelet counts was observed when rabbits receive 3 mg of poly AG i.v. although this polynucleotide which was used because $BLR_{(s)}$ are relatively rich in A and G, has no leukopoietic activity. Finally it may be noted that oral administration of $BLR_{(s)}$ to rabbits (see below) does not cause an initial transcient fall in cell count.

Effect of $BLR_{(s)}$ administered orally. As shown in Fig.11 the effect of orally administered $BLR_{(s)}$ on leukocyte and platelet counts is manifest more slowly and maintained longer that that produced by i.v. injection (compare Fig.10 and 11). Four rabbits were prepared by treatment for 30 days with 100 mg of Endoxan daily and sufficient $BLR_{(s)}$ i.v. at the necessary intervals to ensure survival. Administration of first $BLR_{(s)}$ and then Endoxan were discontinued. Leukocyte counts were then 5,500-5,000/mm^3. After this treatment rabbits cannot spontaneously restore

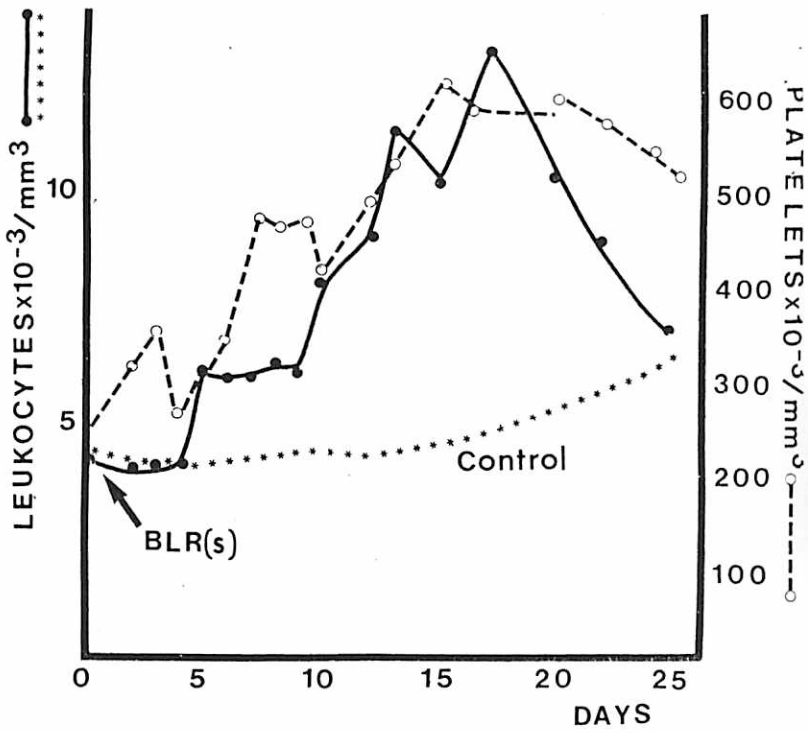

Fig.11 Leukocyte and platelet counts in the rabbit receiving $BLR_{(s)}$ per os. This Fig. shows leukocyte and platelet counts increase in rabbits receiving 20 mg $BLR_{(s)}$ per os . Values represent an average of six rabbits . Initial leukocyte maximum count 3,7000 $\pm$ 100 / mm^3 (a) increased up to 9,250 $\pm$ 2,200 ($p < 0.02$) after BLR administration. Initial platelet maximum count 250,000 $\pm$ 50,000 increased up to 575,000 $\pm$ 25,000 / mm^3 ($p < 0.03$) after BLR administration. (a) values shown are arithmetic mean $\pm$ 1 SD.

leukocyte count within two weeks; a single oral dose of $BLR_{(s)}$ restores both leukocyte and platelet counts to normal levels within about 10 days and maintain these counts at a high level for a further 15 days approximately .

Leukopoietic activity of $BLR_{(s)}$ in methotrexate tested rabbits.

Rabbits given each day 60 mg i.v. doses (for 2 consecutive days) of methotrexate show considerably reduced leukocyte counts (Fig.12). Such rabbits recover a normal leukocyte count if $BLR_{(s)}$ are administered intraveinously , or a little more slowly if they are administered subcutaneously . After cessation of $BLR_{(s)}$ action , leukocyte counts decrease slowly .

$BLR_{(s)}$ activity in platelet formation

Effect on rabbits with a normal platelet count . Platelets which play a major part in blood coagulation are formed in the bone marrow . Since $BLR_{(s)}$ are retained in the bone marrow (fig. 5) it seemed possible that they might have an effect on genesis of platelet blood cells, especially as they act as primers for bone marrow DNA replication . $BLR_{(s)}$ given i.v. or per os cause platelet count to increase in the blood of both Endoxan treated and untreated rabbits . As found for leukocyte counts , cellular regulation bring final platelet counts back to a normal physiological level after $BLR_{(s)}$ stimulation . The results of numerous experiments lead to conclusion that the best results are obtained when $BLR_{(s)}$ are given per os . Increased platelet counts then remain stable for about two weeks .

Effect on rabbits with a decreased platelet count . Daunorubicin (anthracyclin) causes a rapid and spectacular fall of leukocyte and platelet counts in 4 kg rabbits receiving 5 mg of the drug i.v. for four consecutive days only . Toxicity is so high that death occurs on the 6th - 8 th day after the start of the treatment . However , if $BLR_{(s)}$ are given on the 5 th and 7 th day the animal can be saved . Fig. 13 shows the evolution of platelet and leukocyte counts during such an experiment. Daunorubicin treatment reduces platelet

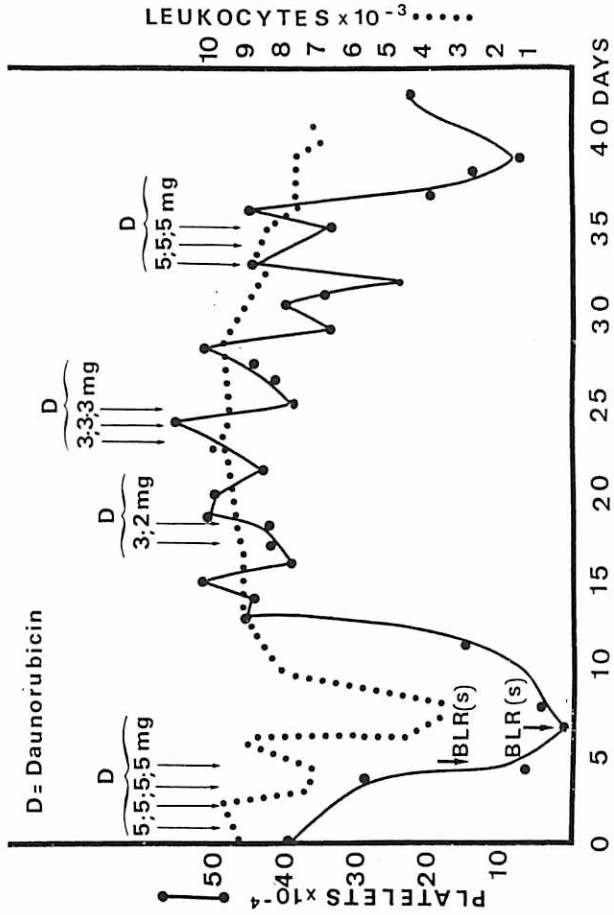

Fig. 12 Effect of $BLR_{(s)}$ on leukocyte formation in rabbits pretreated with methotrexate . Three rabbits each received 60 mg methotrexate (i.v.) for two consecutive days . $BLR_{(s)}$ (5 mg) were injected as shown by the arrow . Leukocyte counts (average values) obtained are shown on the same Fig. i.v. = intraveinous route ; s.c. subcutaneous route . These values are typical for all rabbits treated with

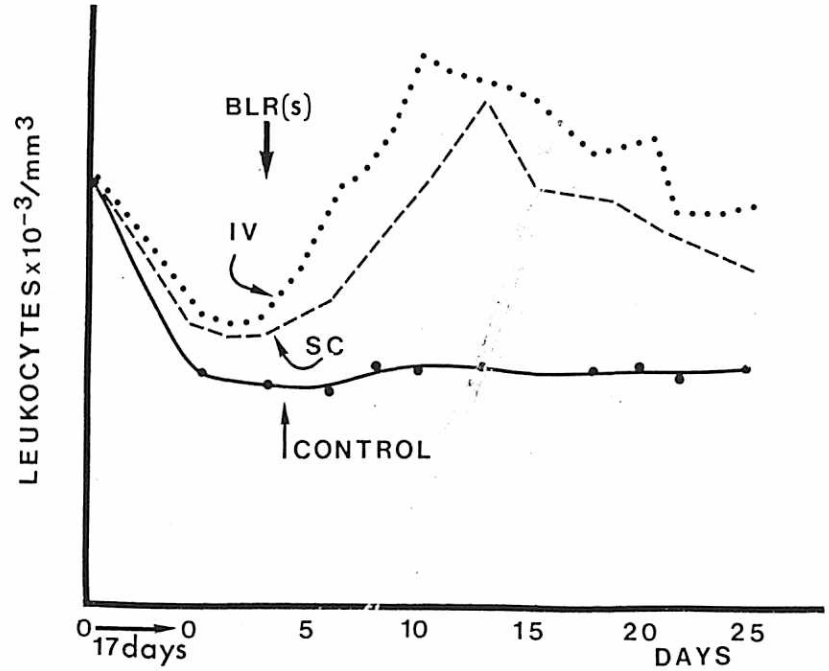

Fig.13 Effect of $BLR_{(s)}$ on platelet count in the rabbit pretreated with high dosages of daunorubicin . A 4 Kg rabbit received i.v. 5 mg Daunorubicin daily for 4 consecutive days . At time shown by arrows, the rabbit received two BLR dosages (5 mg i.v. and 20 mg per os). The leukocyte count which was low in the drug-pretreated animal , was brought back to 10,000 within 48 h . After treating with $BLR_{(s)}$, several doses of Daunorubicin were injected i.v. (see arrows). The same experiment was repeated on another rabbit. Platelet count were performed for both animals. Three control rabbits treated only with Daunorubicin died at the 6th to 9 th day following the first daunorubicin injection .

and leukocyte count from 400,000 and 10,000 to 15,000 and 3,600 respectively. After two doses of $BLR_{(s)}$, (arrows , Fig. 13) leukocyte count returned to normal (10,000/mm^3) in 48 h whereas platelet counts did not reach the initial value of 400,000 until 5 - 6 days later . Subsequent renewed doses of daunorubicin , totalizing 14 mg in 15 days did not cause any significant decrease in the platelet and leukocyte counts . Protection by $BLR_{(s)}$ therefore subsisted and even when 15 mg of daunorubicin was given three weeks after the last $BLR_{(s)}$ dose the fall in platelet counts was much less striking than that observed before administration of $BLR_{(s)}$.

$BLR_{(s)}$ / drug ratio Many chemotherapeutic agents decrease leukocyte and platelet counts and as we show here it is possible to slow down or arrest this effect by appropriate $BLR_{(s)}$ dosage . Thus $BLR_{(s)}$ may make a positive contribution to human chemotherapy and particularly to cancer therapy. The amount of $BLR_{(s)}$ necessary to achieve optimum results can be calculated by giving variable amounts to an animal receiving a constant drug dosage but it must be noted that the value obtained will vary somewhat from animal to animal . Thus for a rabbit receiving 100 mg of Endoxan i.v. daily a $BLR_{(s)}$ - drug ratio of 1 : 20 is normally sufficient to maintain a high leukocyte count for 3 - 4 days . However smaller amounts e.g. a 2 mg dose (instead of 5 mg of $BLR_{(s)}$) ($BLR_{(s)}$ - drug ratio 1 : 50) are sufficient in some cases . Because various drugs e.g. Endoxan , Methotrexate, Daunorubicin damage bone marrow and spleen cells to different extents , optimum $BLR_{(s)}$ - drug ratios must be determined in each case.

Comparison of the effect of $BLR_{(s)}$ and other polynucleotides . Administration of 10 - 20 mg E.coli ribosomal $RNA_{(s)}$ per os to Endoxan treated rabbits causes no noticeable increase in either platelet or leukocyte count. This shows that the high molecular weight r-RNA chains must be degraded to small products ($BLR_{(s)}$) in order to obtained material which can act on platelet and leukocyte formation . Administration of poly AG or poly A by i.v. injection to Endoxan treated rabbits also fails to stimulate leukocyte formation (Fig. 14). The products of deoxyribonuclease digestion of salmon DNA were also found to be inactive with respect to platelet and leukocyte formation in Endoxan treated rabbits.

$BLR_{(s)}$ restore the balance between lymphocytes and polynuclears . In the healthy human , leukocytes contain 60 - 70 % polynuclears and 25 - 40 % lymphocytes whereas in the normal rabbit the amounts of these two types

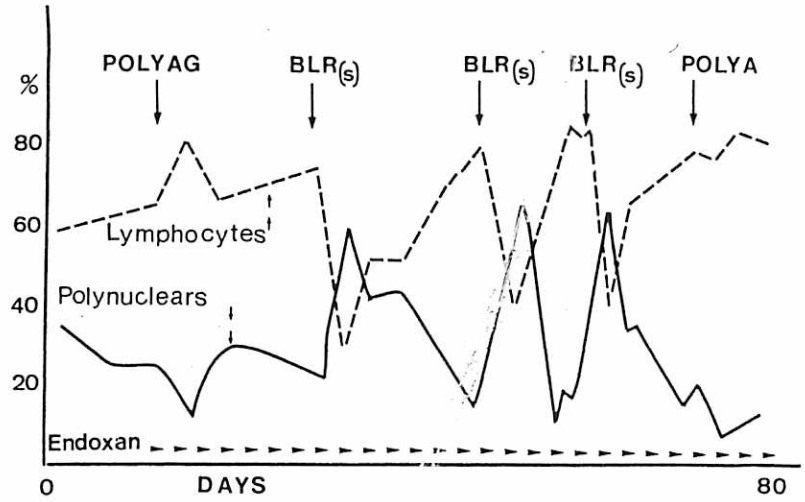

Fig. 14 $BLR_{(s)}$ restore the balance between lymphocyte and polynuclear percentages. A 3 kg rabbit was treated daily with 100 mg Endoxan i.v. for several weeks and periodically with $BLR_{(s)}$. When leukocyte count had fallen and polynuclear and lymphocyte imbalance had appeared , Poly AG (3 mg), $BLR_{(s)}$, (5 mg), Leuco-4 (5 mg), diethyldithiocarbamate (DEDTC)(5 mg), Poly A (5 mg) were given intravenously at the intervals shown on this Fig. The same results were obtained with two other rabbits . The balance-restoring effect of $BLR_{(s)}$ was observed in 10 other rabbits . Initial lymphocyte maximum count 3,530 $\pm$ 322 / mm^3 (a) increased up to 6,100 $\pm$ 520 / mm^3 ($p < 0.02$) after BLR injection . Initial polynuclear maximum count 1,080 $\pm$ 250 / mm^3 increased up to 6,990 $\pm$ 680 ($p < 0.001$) after BLR injection . Cyclophosphamide destroys polynuclears more efficiently than lymphocytes . $BLR_{(s)}$ reestablish first the polynuclear / lymphocyte balance and then normal amounts of both types
(a) values shown are arithmetic mean $\pm$ 1 SD

of cells are usually practically equal . In rabbits , daily Endoxan treatment (100 mg/day/3 kg rabbit), the considerable decrease in leukocyte count seen after 6 - 7 days is accompanied by a large differential decrease in the number of polynuclears which falls from 55 % to 18 % of the total white cell count (Fig.14). Endoxan and other drugs cause a similar effect in man.

When Endoxan treated rabbits are given $BLR_{(s)}$ i.v. polynuclear and lymphocyte counts increase differentially and the number of these cells become practically restored within 4 - 5 h and a normal ratio is fully re-established after 24 h. Six days later the effect of $BLR_{(s)}$ has ended and the Endoxan induced imbalance reappears (Fig.14) but can be again corrected by a further $BLR_{(s)}$ injection . This sequence of events can be repeated again and again . In one Endoxan treated rabbit the effect of $BLR_{(s)}$ on leukocyte formation was compared with that of sodium diethyldithiocarbamate (DEDTC) , poly A , Leuco-4 and poly AG (Fig.14). None of the latter four agents were able to stimulate leukocyte formation or restore a normal lymphocyte / polynuclear ratio .

$BLR_{(s)}$ do not induce tolerance phenomenon in rabbits as Endotoxin does. Since $BLR_{(s)}$ originate from Escherichia coli ribosomal RNA , it appeared essential to distinguish the physiological activity of BLR in leukopoiesis from the effect exhibited by E.coli endotoxin . The Shwartzman reaction (37) , a sensitive test for detection of small amounts of bacterial endotoxin in biological preparations gives in rabbit a positive reaction with 0.2 - µg of bacterial endotoxin or by a few µg of Poly I-Poly C (38). 250 µg of $BLR_{(s)}$ were injected at each of 5 different sites in shaved rabbits epiderm and 16 h later the animals received 5 mg $BLR_{(s)}$ i.v. No trace of inflamation was detected at the inoculation sites for three days. Several $BLR_{(s)}$ preparations were tested on different rabbits and none gave a positive Shwartzman reaction.

It has been well established that daily endotoxin injections to healthy rabbits result in a progressive decrease in the amount of fever and in the transient polynuclear liberation into circulating blood (39)(40). This tolerance state might be induced in rabbits and humans with small doses of endotoxin (1.0 - 5.0 ng/kg) (41)(42) . Rabbits received every 48 h , 1-2 mg BLR/kg i.v., altogether eleven injections. Total white blood cell count was determined at intervals indicated in the legend to Fig15 and rectal temperature taken just before injection and every h thereafter for 6 - 8 h. Firstly, each BLR administration resulted in full leukocyte count increase . Secondly,

the amount of fever produced was the same after the first or the last of the eleven $BLR_{(s)}$ injection (Fig. 15). These data clearly demonstrate failure of tolerance to develop after repeated i.v. administration of BLR , as already suggested by results illustrated in Fig. 10 . To these same rabbits , E.coli endotoxin (1.5 µg / kg) was injected i.v. every 48 h totalizing 7 injections) . The mean peak of polynuclear count declined on successive days in parallel with fever decrease (Fig. 15). Endotoxin tolerant rabbits respond normally to $BLR_{(s)}$: full expression of their leukopoietic activity and undiminished fever response . In rabbits treated with high dosages of Endoxan (100 mg/ 3 kg rabbit), endotoxin (1.0 µg/kg) does not induce transcient polynuclear liberation while under the same conditions 5 mg of $BLR_{(s)}$ induce the restoration of normal leukocyte level with peak response between 24-48 h without inducing tolerance phenomenon. The demonstrations that $BLR_{(s)}$ actively induce not only leukocyte but also platelet formation in rabbits treated with daunorubicin for instance , show that their physiological effects areclearly distinguishable from that exhibited by endotoxin .

Pyrogenicity of various polyribonucleotides. The pyrogenic effect of $BLR_{(s)}$ (1 mg / kg), of Poly AG (1 mg/kg) and of Poly I - Poly C (5 µg/kg) was determined in rabbits injected i.v. in duplicate for each substance. Anal temperature was measured . The mean values (ΔT maximum) were respectively 1.4°C , 1.9°C and 2.0°C and so called temperature period lasted for 4-5 h. The pyrogenic effect of $BLR_{(s)}$ is no higher for 20 mg BLR/kg than for 1 mg BLR/kg, which suggests that the pyrogenic effect of $BLR_{(s)}$ is due to the RNA fragments themselves and not to any possible contaminant. When given per os $BLR_{(s)}$ are not pyrogenic .

$BLR_{(s)}$ activity and animal behaviour . No modification of behaviour was observed in Endoxan-treated or control untreated rabbits which were given i.v. BLR dosages of 0.5 to 20 mg/kg. In 20-22 gr mice injected i.p. with 2 to 20 mg $BLR_{(s)}$, no adverse reactions were noted and the animals survived in normal condition.

$BLR_{(s)}$ and malignant cell development in mice under chemotherapy. The fact that $BLR_{(s)}$ do not stimulate the in vitro replication of DNA from cancerous tissues suggested that in vivo , they could not stimulate malignant

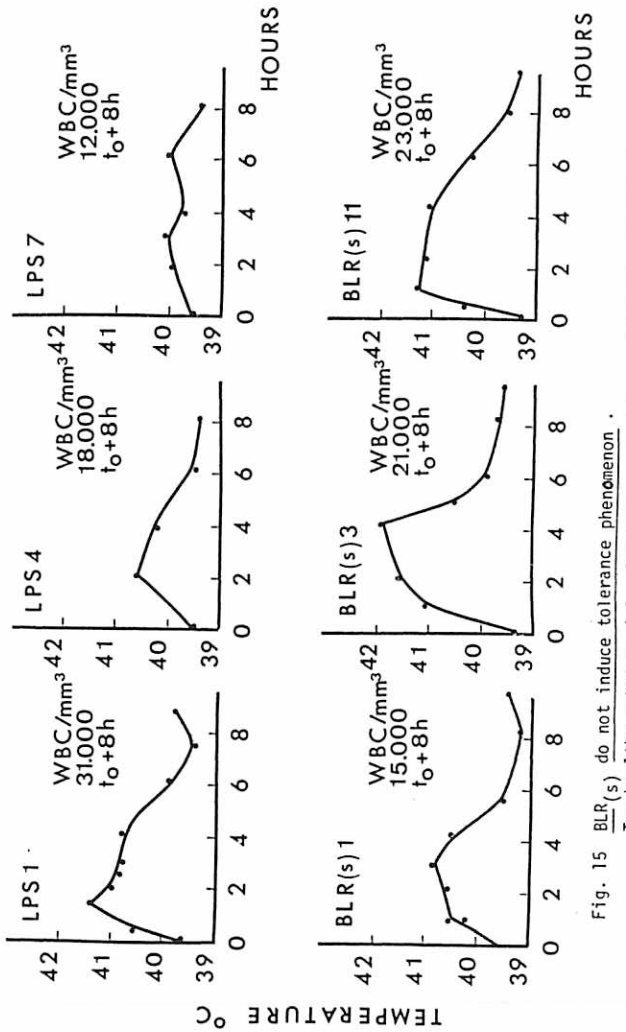

Fig. 15 $BLR_{(s)}$ do not induce tolerance phenomenon .
Two healthy rabbits (3 - 3.5 kg) received 5 µg of LPS (E.coli 011 B4 Endotoxin) i.v. every 48 h . Anal temperature was taken. Average values are presented. White blood cells count was determined at T_o + 8 h (peak value). After rest of three months (there is no more tolerance to endotoxin) these same rabbits received 5 mg of BLR i.v. every 48 h . Anal temperature and white blood cell count (WBC) were determined. WBC : 10 500

of BLRs is evidenced by various observations : 1) the increase of leukocyte and platelet counts produced by BLRs never exceeds normal physiological limits and there is no cumulative effect following repeated dosages; 2) an excess BLR dosage never produces overhigh counts of circulating leukocytes and platelets (this is certainly connected to the finite life span of these blood cells); 3) a normal balance polynuclears and lymphocytes is restored within a few hours following BLR injection; 4) BLRs are not toxic; 5) BLRs do not induce tolerance phenomenon; 6) BLRs have no priming effect on in vitro replication of DNA from various cancerous tissues and stimulate neither cells in mice nor pathological leukocytes.

All these results clearly show that these RNA-fragments (BLRs) do not act by liberating leukocytes and platelets from a reserve pool, but instead act on stem cell DNA replication (stem cell compartment), stem cell division, and also on differentiation in the multiplying compartment, thus giving rise to polynuclears and platelets. Preliminary experiments (Plawecki and al., unpublished) show an increase of myelocyte count in the bone marrow and also in the circulating blood of BLR treated rabbits. The fact that in animals pretreated with BLRs, daunorubicin has a far smaller decreasing effect on platelet and leukocyte counts confirms clearly that a competition exists between BLRs and daunorubicin for stem cells and their derivatives. This competition can also be observed during in vitro DNA replication.

It should be emphasized that rabbits, permanently treated with high cyclophosphamide doses which are lethal for sensitive animals within 8 to 12 days (leukocyte depletion) but receiving a weekly 2 – 6 mg BLR i.v. injection have been kept alive and healthy for 2 – 3 years and showed no ill effect after simultaneous cessation of both the antimitotic drug and BLRs. It should be recalled that once BLRs have achieved their effect on leukocyte and platelet genesis they are degraded by endogeneous nucleases present in animal tissues. BLRs, which are also active per os, do not impede the activity of anti-cancer drugs.

From our four years observations of many rabbits and mice under various conditions, BLRs, which act on humans as they do on animals, may contribute very positively to the cure of leukocyte and platelet deficiencies whether they are induced by chemotherapy (particularly cancer therapy), by various aliments or by genetic defects.

REFERENCES

1. Schekman,R.,W.Wickner, O.Westergaard, O.Brutlag, K.Geider, L.Lbertsch and A.Kornberg. Initiation of DNA Synthesis : synthesis of O X174 Replicative form Requires RNA synthesis Resistant to Rifampicin. Proc.Nat.Acad.Sci. USA 69 : 2691-2695 (1972).

2. Fox,R.N.,J.Mendelsohn, E.Barbosa and M.Goulian. RNA in Nascent DNA from cultured human Lymphocytes. Nature New Biol. 245 : 234-237 (1973).

3. Geider,E.B. and M.Schaller. An RNA transcribed from DNA at the origin of Phage fd single strand to Replicative form conversion. Proc.Nat.Acad.Sci.USA 75, 645-649 (1978).

4. Sugino,A., S.Hirose and R.Okazaki. RNA-linked Nascent DNA Fragments in E. coli .Proc.Nat.Acad.Sci.USA 69 :1863-1867 (1972).

5. Beljanski,M. and M.I.Aaron Da Cunha. Particular Small size and RNA-Fragments from different Origin as Tumor Inducing Agents in Datura stramonium.Molec.Biol.Raports. 2 : 497-506 (1976).

6. Plawecki,M. & M.Beljanski. Synthese in vitro d'un ARN utilisé comme amorceur pour la réplication de l'ADN. C.R.Acad.Sc.Paris série D. 278 : 1413-1416 (1974).

7. Beljanski,M.,M.Beljanski, M.Plawecki, and P.Bourgarel. ARN-fragments, amorceurs nécessaires à la Réplication in vitro de s ADN. C.R.Acad.Sc.Paris Série D. 280 : 363-366 (1975).

8. Evans,A.H. Introduction of Specific drug resistance properties by purified RNA containing fraction. P.N.A.S. 52, 1442-1449 (1964).

9. Beljanski,M. M.S.Beljanski, P.Manigault and P.Bourgarel. Transformation of A.tumefaciens into a non-oncogenic species by an E.coli RNA. P.N.A.S. USA 69 : 191-195 (1972).

of BLRs is evidenced by various observations : 1) the increase of leukocyte and platelet counts produced by BLRs never exceeds normal physiological limits and there is no cumulative effect following repeated dosages; 2) an excess BLR dosage never produces overhigh counts of circulating leukocytes and platelets (this is certainly connected to the finite life span of these blood cells); 3) a normal balance polynuclears and lymphocytes is restored within a few hours following BLR injection; 4) BLRs are not toxic; 5) BLRs do not induce tolerance phenomenon; 6) BLRs have no priming effect on in vitro replication of DNA from various cancerous tissues and stimulate neither cells in mice nor pathological leukocytes.

All these results clearly show that these RNA-fragments (BLRs) do not act by liberating leukocytes and platelets from a reserve pool, but instead act on stem cell DNA replication (stem cell compartment), stem cell division, and also on differentiation in the multiplying compartment, thus giving rise to polynuclears and platelets. Preliminary experiments (Plawecki and al., unpublished) show an increase of myelocyte count in the bone marrow and also in the circulating blood of BLR treated rabbits. The fact that in animals pretreated with BLRs, daunorubicin has a far smaller decreasing effect on platelet and leukocyte counts confirms clearly that a competition exists between BLRs and daunorubicin for stem cells and their derivatives. This competition can also be observed during in vitro DNA replication.

It should be emphasized that rabbits, permanently treated with high cyclophosphamide doses which are lethal for sensitive animals within 8 to 12 days (leukocyte depletion) but receiving a weekly 2 – 6 mg BLR i.v. injection have been kept alive and healthy for 2 – 3 years and showed no ill effect after simultaneous cessation of both the antimitotic drug and BLRs. It should be recalled that once BLRs have achieved their effect on leukocyte and platelet genesis they are degraded by endogeneous nucleases present in animal tissues. BLRs, which are also active per os, do not impede the activity of anti-cancer drugs.

From our four years observations of many rabbits and mice under various conditions, BLRs, which act on humans as they do on animals, may contribute very positively to the cure of leukocyte and platelet deficiencies whether they are induced by chemotherapy (particularly cancer therapy), by various aliments or by genetic defects.

REFERENCES

1. Schekman,R.,W.Wickner, O.Westergaard, O.Brutlag, K.Geider, L.Lbertsch and A.Kornberg. Initiation of DNA Synthesis : synthesis of O X174 Replicative form Requires RNA synthesis Resistant to Rifampicin. Proc.Nat.Acad.Sci. USA 69 : 2691-2695 (1972).

2. Fox,R.N.,J.Mendelsohn, E.Barbosa and M.Goulian. RNA in Nascent DNA from cultured human Lymphocytes. Nature New Biol. 245 : 234-237 (1973).

3. Geider,E.B. and M.Schaller. An RNA transcribed from DNA at the origin of Phage fd single strand to Replicative form conversion. Proc.Nat.Acad.Sci.USA 75, 645-649 (1978).

4. Sugino,A., S.Hirose and R.Okazaki. RNA-linked Nascent DNA Fragments in E. coli .Proc.Nat.Acad.Sci.USA 69 :1863-1867 (1972).

5. Beljanski,M. and M.I.Aaron Da Cunha. Particular Small size and RNA-Fragments from different Origin as Tumor Inducing Agents in Datura stramonium.Molec.Biol.Raports. 2 : 497-506 (1976).

6. Plawecki,M. & M.Beljanski. Synthese in vitro d'un ARN utilisé comme amorceur pour la réplication de l'ADN. C.R.Acad.Sc.Paris série D. 278 : 1413-1416 (1974).

7. Beljanski,M.,M.Beljanski, M.Plawecki, and P.Bourgarel. ARN-fragments, amorceurs nécessaires à la Réplication in vitro de s ADN. C.R.Acad.Sc.Paris Série D. 280 : 363-366 (1975).

8. Evans,A.H. Introduction of Specific drug resistance properties by purified RNA containing fraction. P.N.A.S. 52, 1442-1449 (1964).

9. Beljanski,M. M.S.Beljanski, P.Manigault and P.Bourgarel. Transformation of A.tumefaciens into a non-oncogenic species by an E.coli RNA. P.N.A.S. USA 69 : 191-195 (1972).

10. Beljanski,M and M.Plawecki . Transforming RNA as a template directing RNA and DNA synthesis in bacteria. In the Role of RNA in Reproduction and Development Niu M.C. and Segal, S.J. Eds. North Holland Publ.Co. 199-224 (1973).

11. Mishra,N.C., M.C.Niu and E.L. Tatum . Induction by RNA of Inositol Independence in Neurospora crassa . Proc.Natl.Acad.Sci. USA 72 , 642-645 (1975)

12. Tung, T.C. and Niu, M.C. Nucleic Acid-Induced transformation in Goldfish. Scientia Sinica XVI : 366-384 (1973)

13. Tung, T.C. and M.C.Niu . Transmission of the nucleic acid induced character , caudal fin , to the offspring in Goldfish . Scientia Sinica XVIII : 223-228 (1975).

14. Fishman,M. The Role of macrophage RNA in the immune response. In The Role of RNA in Reproduction and Development. Niu,M.C. and Segal,S.J. eds. North Holland Publ.Co. 127-136 (1973)

15. Niu,M.C. Cellular Mechanism in differentiation and Growth. Ed.Rudnick . Princeton Univ. Press. 155-171 (1956)

16. Niu,M.C. Thymus Ribonucleic acid and Embryonic differentiation. Proc.Natl.Acad. Sci. USA 44 : 1264-1274 (1958)

17. Lee,H and M.C.Niu . Studies on Biological potentiality of testis-RNA. I.Induction of axial structures in whole and excised chick blastoderms. In The Role of RNA in Reproduction and Development . Niu,M.C. and Segal,S.J. Eds. North Holland Publ. Co. 137-154 (1973)

18. Deshpande,A.K., S.B.Jakowlew , Arnold,H, P.A.Crawford, M.A.Q.Siddiqui. A novel RNA affecting embryonic gene functions in early chick blastoderm . J.Biol. Chem. 252 : 6521-6527 (1977)

19. Beljanski,M, M.I.Aaron Da Cunha, M.S.Beljanski, P.Manigault, and P.Bourgarel. Isolation of the Tumor Inducing RNA from oncogenic and non-oncogenic Agrobacterium tumefaicens . Proc. Natl. Acad.Sci. USA 71 : 1585-1589 (1974)

20. Beljanski,M. , L.Chaumont , C.Bonissol and M.S.Beljanski. ARN fragments , Inhibiteurs de la multiplication des virus du Fibrome de Shope et de la Vaccine.. C.R.Acad.Sc. Paris Série D , 280 : 783-789 (1975)

21. Beljanski,M., M.Plawecki, P.Bourgarel and M.S.Beljanski. Nouvelles substances (RLB) actives dans la leucopoiese et la formation des plaquettes. Bull.Acad. Nat. Med. 162 : 475-481 (1978)

22. Le Goff,L. and M.Beljanski. Stimulation de l'induction ou inhibition du developpement des tumeurs de Crown gall par des ARN fragments U_2 . Interférence de l'auxine . C.R.Acad.Sci. Paris Série D 288 , 147-150 (1978)

23. Schlager,S.I., R.E.Paque and S.Dray. Complete and Apparently specific local tumor Regression using syngeneic or xenogeneic Tumor-Immune RNA extracts. Cancer Res. 35 : 1907-1914 (1975)

24. Pottathil,R. and H.Meier. Antitumor Effects of RNA isolated from murine Tumors and Embryos. Cancer Res. 37 : 3280 - 3286 (1977)

25. Pardee, A.B., F.Jacob and J.Monod. The Genetic Control and Cytoplasmic Expression of "Inductibility" in the Synthesis of β-Galactosidase by Escherichia coli J.Mol.Biol. 1 : 165-178 (1959)

26. Beljanski,M. , P.Bourgarel and M.S.Beljanski. Drastic Alteration of Ribosomal RNA and Ribosomal Proteins in Showdomycin-resistant Escherichia coli . Proc. Natl. Acad. Sci. USA 68 : 491-495 (1971)

27. Shine,J. and L.Dalgarno. Terminal-Sequence Analysis of Bacterial Ribosomal RNA. Correlation between the 3'-terminal Polypyrimidine. Sequence of 16 S RNA and Translational Specificity of the Ribosome . Eur.J.Bioch. 57 : 221-230 (1975).

28. Beljanski,M., P.Bourgarel and M.S.Beljanski. Showdomycine et Biosynthèse d'un ARN non complémentaire de l'ADN. Inst.Pasteur 118 : 253-276 (1970)

29. Lowry,H. , N.J.Rosebrough, A.L.Faar and R.J.Randall. Protein Measurement with Phenol Reagent . J.Biol.Chem. 193 : 265-269 (1951)

30. Burton,K. A Study of the Conditions and Mechanism of the Diphenylamine Reaction for the Colorimetric estimation of Deoxyribonucleic Acid. Biochem. J . 62 : 315-323 (1956)

31. Björk,G.R. and I.Svensson . Analysis of Methylated Constituents from RNA by Thin Layer Chromatography . Biochem. Biophys. acta 138 : 430-433 (1967)

32. Hori,M. Chromatography of Nucleic Acid Components on Dowex 1 Column by the use of an Automatic Recording System. In Methods in Enzymology. Nucleic Acids Part A. Ed.Grossman,L. and Moldave,K. Academic Press XII 381-390 (New York) (1967).

33. Schildkraut,C.L. and J.J.Mai . Fractions of HeLa DNA depending on their Content of Guanine + cytosine . J. Mol.Biol. 46 : 305-312 (1969)

34. Beljanski,M. and M.S.Beljanski . RNA-Bound Reverse Transcriptase in Escherichia coli and in vitro Synthesis of a Complementary DNA. Biochemical Genetics 12 : 163-180 (1974)

35. Bessis,M. Le Sang et la Lymphe . Encyclopédie Française (sous la Direction du Professeur Grassé). Librairie Larousse IV : 229-241 (1960)

36. Hill,D.L., W.R.Jr.Laster and R.F.Struck. Enzymatic Metabolism of Cyclophosphamide and Nicotine and Production of a Toxic cyclophosphamide metabolite. Cancer Res. 32 : 658 - 665 (1972)

37. Shwartzman,G. Phenomenon of Local Tissue Reactivity and its Immunological, Pathological and clinical Significance . New York : P.B. Hoeber Incorp. 1-37 (1937)

38. Lindsay,H.L., P.W.Trown. J.Brandt and M.Forbes. Pyrogenicity of Poly I-Poly C in rabbits . Nature 223 : 717-718 (1969)

39. Smith,W.W., I.M.Alderman and J.Cornfield . Granulocyte release by endotoxin in normal and irradiated mice. Am.J.Physiol. 201 : 396-402 (1961)

40. Fukuda,T. and O.Matsumato. Endogenous Factors concerning the febrile and the leukocytic response to bacterial endotoxin in relation to the adrenal cortex. Jap. J. Physiol. 9 : 274-281 (1959)

41. Mechanic,R.C., E.Frei III , M.Landy and W.W.Smith. Quantitative studies of human leukocytic and febrile response to single and repeated doses of purified bacterial endotoxin. J.Clin.Invest. 41 : 162-172 (1962)

42. Dale,C.D., A.S.Fauci , D.P.IV Guerry and S.M.Wolff. Comparison of Agents Producing a Neutrophilic Leukocytosis in Man. J.Clin.Invest 56 : 808-813 (1975)

43. Henderson,E.S. The Granulocytopenic Effects of cancer chemotherapeutic agents in "Drugs and Hematologic Reactions ". Eds N.V.Dimitrov and J.H.Nodine. The Twenty ninth Hahnemann Symposium . 18 : 207-220 , 1974. Grune and Stratton New York , London