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Inhibition of Cell Proliferation by D-Ribose and Deoxy-D-ribose (42172)

MARINA MARINI,* GIOVANNA ZUNICA,* AND CLAUDIO FRANCESCHI†

*Institute of Histology and General Embryology, School of Medicine, Via Belmeloro, 8-40126 Bologna, and †Institute of General Pathology, School of Medicine, Via L. Loredan, 16-35100 Padua, Italy

Abstract. D-Ribose and deoxy-D-ribose inhibited DNA, RNA, and protein synthesis in a wide variety of cells (dividing and nondividing, normal and neoplastic, freely floating and substrate adhering, human and murine) at concentrations at which other monosaccharides have little or no effect. Inhibition was irreversible and proportional to the sugar concentration and time of contact. However, the first effects were seen only after 24 hr of incubation and progressed slowly to cell death. Whether the two sugars share the same mechanisms of action is not known. In any case, they deeply derange metabolic processes in both dividing and nondividing cells.

It is now accepted that many cell functions are mediated by the carbohydrate moieties present on the cell surface, and that the addition of monosaccharides to the culture medium specifically modifies some of these functions. Apart from a few studies on fibroblastoid cell lines (1), most work has been performed using lymphocytes and macrophages where it is likely that the addition of sugars affects cell–cell interactions and responsiveness to chemotactic factors (2–12).

We previously found (13) that, at a concentration of 50 m*M*, a number of sugars partially inhibited lymphocyte proliferation in allogeneic mixed lymphocyte cultures, while not affecting PHA-induced [^{3}H]TdR incorporation. Galactosamine, glucosamine, D(−)-ribose (Rib) and 2-deoxy-D-ribose (dRib), instead, inhibited both mixed lymphocyte cultures and PHA-induced proliferation by 90% or more.

The toxicity of amino-sugars is now well accepted (14, 15); however, the inhibition exerted by Rib and dRib, two of the commonest sugars found in biological molecules, was somewhat unexpected and prompted us to study it in more detail. It is known that the two pentoses are utilized for the formation of nucleotides through the salvage pathways; since they can be metabolized to form lactate and yield ATP (16), it is inappropriate to consider them as metabolic inhibitors. Two papers published recently (11, 17) have reported data on the action of Rib on lymphocytes, but the possible mechanisms of action were not explored.

In the present paper we have demonstrated that the inhibition exerted by Rib and dRib is not limited to lymphoid cells but is a more generalized phenomenon affecting a variety of cells; the two sugars block DNA, RNA, and protein synthesis simultaneously to an extent which is roughly proportional to the dose and time of contact; their action ultimately results in cell death.

Materials and Methods. 2-Deoxy-D-ribose and D(−)-arabinose (Ara) were purchased from Sigma, D(−)-ribose from Calbiochem Corporation and from Serva. To determine whether the sugars used contained impurities, which could be responsible for their biological effects, 250 μg of each sugar was subjected to thin-layer ascending chromatography using silica gel-coated plates (DC-Fertigplatten, Kieselgel 60, Merck) using the following solvent systems: (i) butanol:acetic acid:water (3:1:1); (ii) ethyl acetate:pyridine:water (3:1:3); and (iii) ethanol:butanol:pyridine:acetic acid:water (100:10:10:3:30). The plates were then stained by spraying them with *p*-anisaldehyde (Eastman Kodak Co.) and heating at 120°C.

Lymphocyte cultures. Peripheral blood from healthy volunteers was collected aseptically; lymphocytes were separated by centrifugation in MSL (Eurobio, Paris), extensively washed with RPMI 1640 medium (Eurobio, Paris) and resuspended at the concentration of 10^6 cells/ml in RPMI 1640 supplemented by 10% heat-inactivated pooled human AB serum, 2 m*M* L-glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml).

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Spleens from 3-month-old C57/BL mice were obtained aseptically and forced through a fine-mesh wire net with the aid of a rubber policeman. Single-cell suspensions were washed in RPMI 1640 and adjusted to a concentration of $4 \cdot 10^6$ cells/ml in serum-supplemented RPMI 1640 medium. Cell suspension (0.1 ml) was seeded in each well of a 96-well flat-bottomed culture plate (Falcon) and, unless otherwise specified, 0.1 ml of culture medium containing the mitogen was added. The final concentrations of the mitogens were PHA-P (Difco): 1 μl/ml; Con A (Miles Lab.): 10 μg/ml; PWM (Grand Island Biological Co.): 10 μl/ml; and LPS (Difco, Detroit) from *Escherichia coli* 055B5: 10 μg/ml. LPS was dissolved in phosphate-buffered saline, boiled for 2 hr, and stored at room temperature. Unstimulated controls were always performed. For experiments in which labeled leucine was used, the cells were suspended in RPMI 1640 without leucine.

Cultures were incubated 72 hr in a humidified atmosphere of 5% CO_2 in air at 37°C; at the 66th hr, 0.5 μCi of [^{3}H]TdR (Amersham, spec act 5 Ci/mmole), 0.25 μCi of [^{3}H]uridine (U; Amersham, spec act 58 Ci/mmole) and/or 0.250 μCi of [^{14}C]Leu (New England Nuclear, spec act 339 mCi/mmole) were added to each well. Total cell radioactivity was evaluated by extensively washing the cells and digesting them with Beckman BTS tissue solubilizer before counting. Acid-precipitable radioactivity was evaluated in one of two ways according to the marker. When cells were labeled with [^{3}H]TdR or [^{3}H]U, plates were frozen overnight at the end of the incubation, and then processed with the aid of a multiple-cell harvester (Skatron, Oslo); finally, the filters were solubilized and counted in a Beckman EP liquid scintillation mixture. When cells were labeled with [^{14}C]Leu, alone or in combination with [^{3}H]TdR or [^{3}H]U, one-half of the medium of each well was carefully pipetted off and substituted with an equal volume of 0.2 *N* KOH. One volume of 20% cold trichloroacetic acid was then added. The content of each well was individually filtered onto Whatman GF/C filters; each filter was extensively washed with 5% trichloroacetic acid and counted in Beckman NA liquid scintillation mixture with ethylene glycol monomethyl ether; this procedure had been previously described (18).

Triplicate wells were always prepared, and counts were averaged. Most results have been expressed as a percentage of control cultures, i.e., cpm (experiment) × 100/cpm (control). Control cultures received the same treatment as experimental ones, except that the sugar solution was replaced by the same volume of RPMI 1640 medium. In the experiments involving the addition or withdrawal of sugars at different times, control cultures were prepared for each experimental time.

Other cell cultures. HL60, U937, and NALM-6 human cell lines were cultured in Falcon flasks with RPMI 1640 supplemented by 10% fetal calf serum. Cultures were split every 2–3 days. For the experiments described here, a sufficient number of cells was recovered from the bottles irrespective of their original crowding. Cells were washed, their concentration was adjusted to 10^6 cells/ml, and 96-well plates were prepared as for lymphocyte cultures.

Bone marrow cells were obtained from femurs of 3-month-old C57/BL mice, washed, and adjusted to the concentration of $4 \cdot 10^6$ cells/ml in Dulbecco's modified Iscove's medium (Gibco) supplemented by 10% horse serum. 0.5 ml of cell suspension was seeded in each well of a 24-well culture plate; after 1 week, only fibroblast-like cells were still present (19) and actively dividing, all other cells having died on the third to fourth day. Half the volume of the well was then substituted by fresh medium, containing appropriate concentrations of the sugars, which were omitted in controls; the cultures were then continued for 72 hr and labeled during the last 6 hr of culture. For the experiment presented in Section 4.1 of Results, 2-week-old cultures grown in 35-mm petri dishes were utilized. Fibroblasts had formed a semiconfluent monolayer and were not dividing any more.

Newborn rat myoblasts were obtained by trypsin dissociation of leg muscles and grown in plastic Falcon bottles in Dulbecco's modified Eagle's medium supplemented with 10% horse serum and 2% chick embryo extracts according to the method described by Carraro *et al.* (20). Fifty millimolar Rib, dRib, or Ara was added at the onset of the cultures and was

omitted in controls. The cultures were microscopically examined daily; on Days 5 and 8 they were sacrificed, and myosin patterns examined by SDS–polyacrylamide gel electrophoresis as described in Ref. (20).

Separation of T cells by rosetting. The technique employed has been described by Franceschi *et al.* (21).

Trypan blue exclusion test. Medium (350 μl) containing $3 \cdot 10^5$ lymphocytes was added to 150 μl of a 0.4% trypan blue solution in saline, incubated in test tubes at 37°C for 8 min and examined in an hemocytometer without further dilution. The number of viable cells was related to the total cell population. Since the classification of cells within the categories of "viable" and "not viable" leaves wide margins of subjectivity, the number of viable cells may have been overestimated.

In-the-well removal and substitution of the medium. The relatively small number of cells present in each well and the fragility of the activated lymphocytes made the use of the centrifuge unsuitable for washing away the medium present in the well to substitute it with one devoid of sugars, when and as required by the experimental protocol. In this case, one-half of the medium was gently pipetted off taking care not to disturb the cells lying on the bottom. An equal volume of sugar-free medium maintained at room temperature was then added and the whole procedure repeated four more times within a few minutes. This method reduced the sugar concentration to 1/32 of the original one, i.e., to levels devoid of any detectable effect on the cells, as proved by preliminary experiments.

Quantitative determination of DNA content. A minimum of 10^6 cells were pelletted in a test tube; 1 ml of cold 0.6 *N* perchloric acid was added and precipitation was allowed to occur at 0°C for 10 min. The tubes were spun at 3500 rpm, 10 min at 0°C, and the supernates discarded. One milliliter of 0.3 *N* KOH was added, and the precipitate was hydrolyzed 60 min at 37°C with occasional stirring. The tubes were cooled, then 0.15 ml of 6 *N* perchloric acid was added; after 10 min more, the tubes were spun and the supernate containing ribonucleotides were discarded. The precipitates were washed with 1 ml of 0.6 *N* perchloric acid, then 1 ml of 7% perchloric acid was added, and DNA was hydrolyzed for 20 min at 70°C. After cooling, tubes were spun down again and the supernates utilized for either diphenylamine color development or direct assessment of the absorption at 260 nm. The presumptive number of cells was calculated on the basis of a standardization curve that attributed an average DNA content of 8 μg/10^6 cells.

Results. *(1) Purity controls.* The purity of the utilized sugars was checked by thin-layer ascending chromatography developed with three solvent systems as described under Materials and Methods. Upon staining with *p*-anisaldehyde, only one spot was detected for each sugar showing that other sugars were not contaminating the commercial preparations of Rib, dRib, and Ara. The presence of a protein contaminant was ruled out by measuring the absorption at 260 and 280 nm of aqueous 500 m*M* solutions of the sugars.

(2) Inhibition of [^{3}H]TdR incorporation in stimulated lymphocytes. Rib and dRib were added to cultures of normal human lymphocytes at the same time as the mitogen—PHA, Con A, or PWM. [^{3}H]TdR incorporation was evaluated on the third day and found to be inhibited by the two sugars in a dose-dependent fashion, as shown in Fig. 1. Rib inhibition increased gradually with increasing concentrations of the sugar; the hemidose was approximately 30 m*M* and maximum inhibition—near 100%—was reached at 50 m*M*. dRib inhibition grew more steeply, the hemidose was approximately 2 m*M* and a full inhibition was reached at 10 m*M*.

No substantial difference could be observed in the different mitogens employed, and the different experimental results were very reproducible.

When Rib and dRib were added to normal murine splenocytes, stimulated by PHA, Con A, or LPS, an inhibition of [^{3}H]TdR incorporation was also observed; dose–response determinations showed a more marked inhibition of murine splenocytes at lower concentrations of the monosaccharides; again, no difference in the mitogen could be observed. Results are shown in Fig. 2.

On the other hand, D(−)-arabinose, a D(−)-ribose isomer, did not substantially affect [^{3}H]TdR incorporation by human or murine-

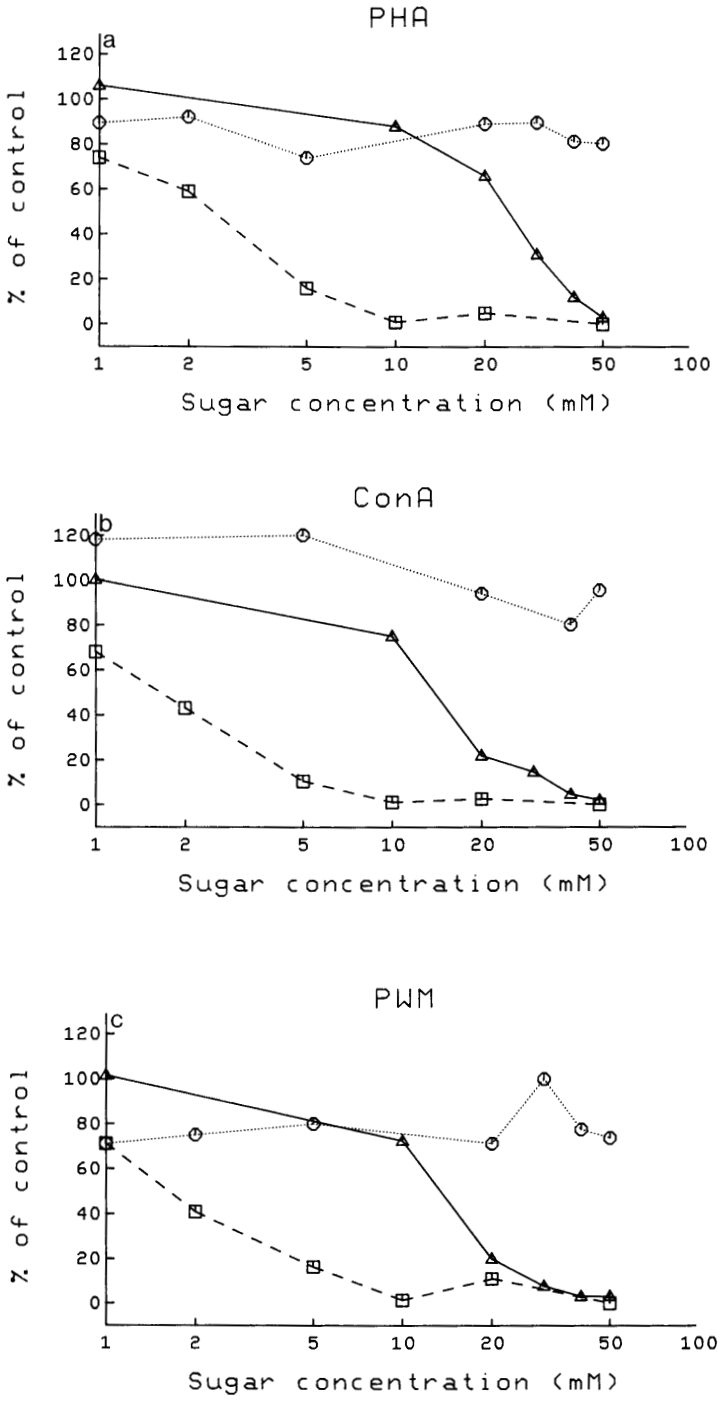

FIG. 1. [^{3}H]TdR incorporation of normal human lymphocytes stimulated with different mitogens. The sugars were added at the beginning of the 72-hr cultures. Controls did not receive any sugar. Data are the average of three independent experiments, each made in triplicate. SEM never exceeded 10. △, Rib; □, dRib; ○, Ara.

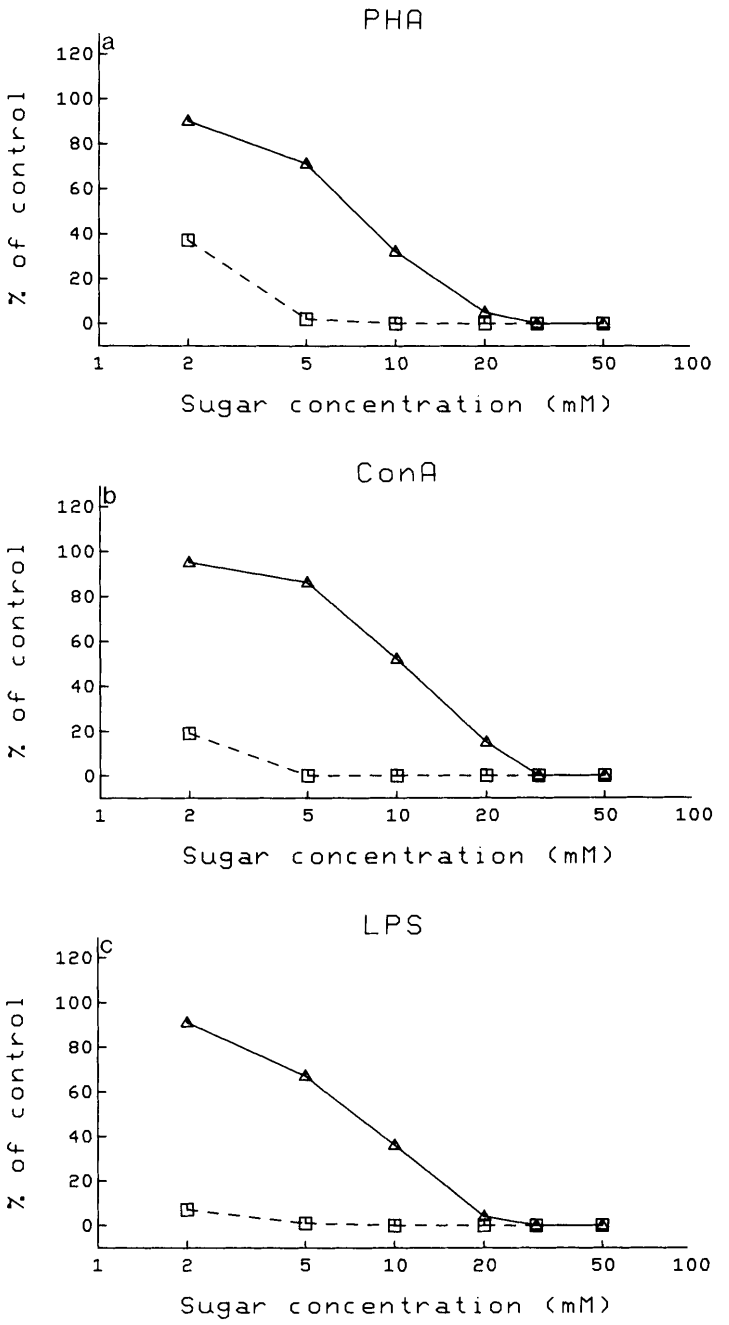

FIG. 2. [^{3}H]TdR incorporation of normal C57/BL splenocytes stimulated with different mitogens. The sugars were added at the beginning of the 72-hr cultures. Controls did not receive any sugar. Data are the average of triplicate wells of one experiment. The experiment was repeated once with similar results. △, Rib; □, dRib; ○, Ara.

stimulated lymphocytes, and was therefore used in most experiments reported here as a control sugar.

Since PHA and Con A are known to stimulate the blastization of T lymphocytes, whereas PWM and LPS are mostly B cell mi-

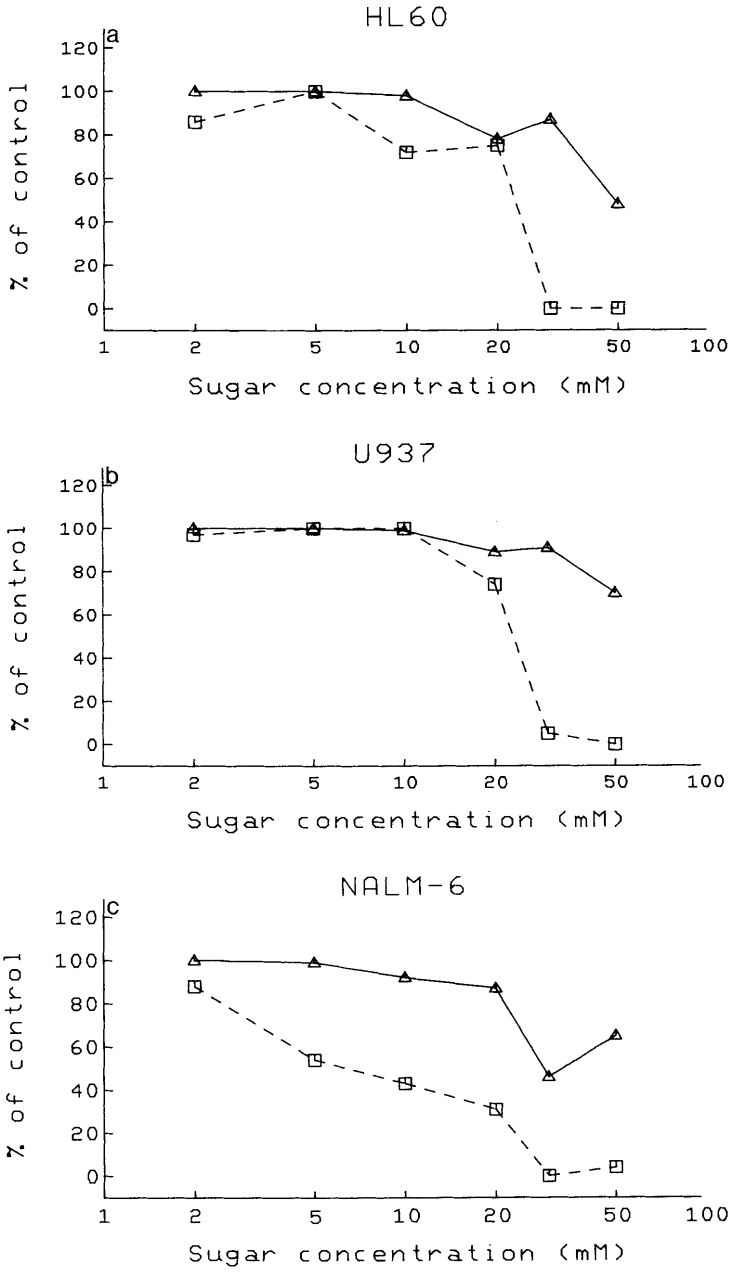

FIG. 3. [^{3}H]TdR incorporation of cell cultures growing spontaneously. The sugars were added 72 hr before the sacrifice of the cultures. Controls did not receive any sugar. Data are the average of three independent experiments, each made in triplicate. SEM was occasionally high. △, Rib; □, dRib.

togens, it is evident that both lymphoid subpopulations are affected by Rib and dRib. This was confirmed by purifying human T lymphocytes by rosetting and stimulating them with PHA in the presence of the two sugars. Results (not shown) fully overlapped with those obtained with unseparated lymphocytes.

(3) Inhibition of [^{3}H]TdR incorporation in other cell types. Since the inhibition exerted by the monosaccharides had proved to be in-

dependent of the mitogenic stimulus, the study was extended to spontaneously and actively dividing cells. Cells studied for this purpose were normal murine marrow-derived fibroblasts, the human myeloid cell line HL60, the human histiocytic cell line U937 and the human B-lymphoid cell line NALM-6. These cells were cultured for 72 hr in the presence of different concentrations of Rib and dRib, then [^{3}H]TdR incorporation was evaluated. All four cell cultures proved to be inhibited by the two sugars, but at higher concentrations than lymphocytes. Results for HL60, U937, and NALM-6 are presented in Fig. 3. The extent of the inhibition—though dose-dependent—varied for the different experiments; this might be correlated to differences in the growth rate of the single cultures.

(4) Effect on the viability of the cells. Four sets of experiments were performed to assay the viability of not dividing cells cultured in the presence of either Rib or dRib.

(4.1) Morphological evaluation of fibroblast and myoblast cultures. Semiconfluent bone marrow derived fibroblast cultures grown in dishes were examined under the inverted microscope after various periods of contact with 50 m*M* Rib or 10 m*M* dRib. At 48–96 hr after the addition of the sugars, a considerable number of cells appeared rounded up and detached from the plate, a recognized sign of cell sufferance. Twenty-four hours later, massive cell lysis had occurred throughout the plate, and the few remaining cells could not be rescued by substituting the sugar-containing medium with fresh medium without sugar. Control plates appeared unchanged.

At the onset of the culture, 50 m*M* Rib, dRib, or Ara was added to myoblast cultures from rat fetus. Forty-eight hours later, cultures which received 50 m*M* dRib displayed a total destruction of the cells. Other plates were cultured for 8 days more; no difference could be observed between those which received either Rib or Ara and the controls (no sugar added), with respect to the morphology, the spontaneous contractility, and the myosin patterns.

(4.2) Trypan blue exclusion. Unstimulated human lymphocytes were incubated in multiwell dishes to which either 30 or 50 m*M* Rib, 2 or 10 m*M* dRib, or 50 m*M* Ara was added. Triplicate wells were pooled at different times and viable cells assessed by the dye exclusion method. Ninety-six hours after the addition of the sugars, considerable cell death had occurred in the plates that had received Rib and dRib, but the first effects were recognizable at the 72nd hr. Results are presented in Table I.

TABLE I. EFFECT OF Rib AND dRib ON THE VIABILITY OF UNSTIMULATED LYMPHOCYTES[a]

Addition	Time of contact with the sugars (hr)					
	1½	6	24	48	72	96
None	100	99	98	93	93	97
30 m*M* Rib	100	99	96	79	91	82
50 m*M* Rib	98	98	96	88	85	61
2 m*M* dRib	99	96	97	92	92	86
10 m*M* dRib	99	95	95	83	79	26
50 m*M* Ara	96	96	94	96	91	86

[a] The viability was evaluated with the method of trypan blue exclusion. The values represent the viability (% of total cells counted) of a pool of cells from three wells. The experiment was repeated once with similar results.

(4.3) [^{3}H]TdR incorporation after stimulation of sugar-treated lymphocytes. Unstimulated normal human lymphocytes were incubated in the presence of Rib, dRib, or Ara. At different times, the sugar-containing medium was carefully substituted (see Methods) with PHA-containing medium without sugar; cultures were continued for a further 72 hr and [^{3}H]TdR incorporation during the last 6 hr of culture was evaluated. Results are presented in Fig. 4. Even 24 hr of contact with 10 m*M* dRib was enough to fully prevent subsequent [^{3}H]TdR incorporation, whereas 48 hr were necessary to fully evidence the inhibition by 2 m*M* dRib and 30 or 50 m*M* Rib.

(4.4) Reversibility of the inhibition on resting lymphocytes. After 24 or 48 hr of incubation with the inhibiting monosaccharides, the medium was substituted to get rid of the sugars and the unstimulated lymphocytes were allowed a further 24-hr relief before the stimulation with PHA. No substantial increase in [^{3}H]TdR incorporation was observed in comparison with lymphocytes which were not allowed 24 hr of relief.

(5) Effect on in vivo DNA, RNA, and protein synthesis. The effect of the sugars on RNA and protein synthesis was studied by labeling lymphocyte cultures with [^{3}H]U and [^{14}C]Leu, alone or in combination ([^{3}H]TdR + [^{14}C]Leu

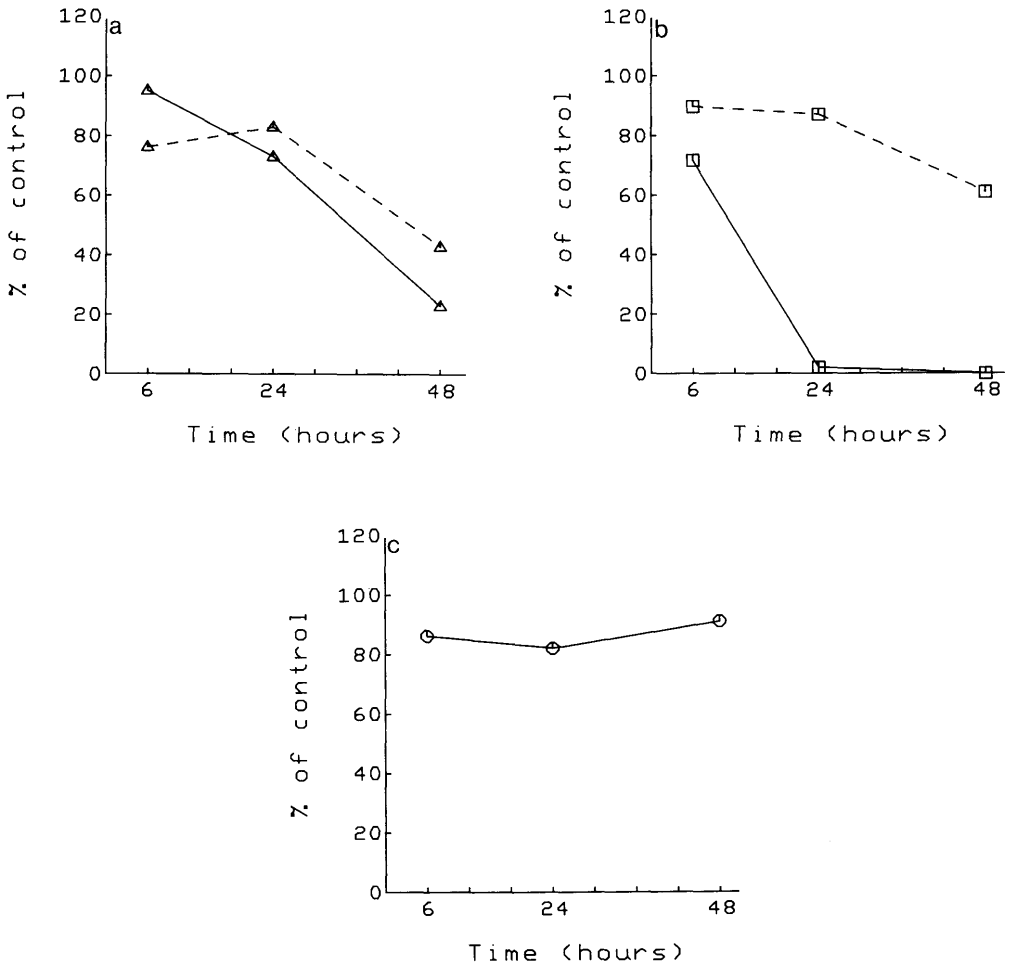

FIG. 4. [^{3}H]TdR incorporation of normal human lymphocytes treated with Rib, dRib, or Ara. The sugars were added to unstimulated lymphocytes. At the desired times, sugars were removed and the cells were stimulated with PHA for 72 hr. Controls did not receive any sugar. Data are the average of three independent experiments, each made in triplicate. SEM never exceeded 10. (a) △ — △, 50 m*M* Rib; △ --- △, 30 m*M* Rib. (b) □ — □, 10 m*M* dRib; □ --- □, 2 m*M* dRib. (c) ○, 50 m*M* Ara.

and [^{3}H]U + [^{14}C]Leu). Label uptakes were found to be parallel in all examined conditions. Sugars were found to inhibit DNA and protein synthesis to approximately the same extent, while RNA synthesis was slightly less inhibited. Results are shown in Table II.

These double-label experiments also constitute an indirect proof that the observed inhibition of [^{3}H]TdR uptake is a true index of a block in cell proliferation. To rule out the possibility that the failed incorporation of the DNA precursor might be due to competition in the entry of the label, Rib and dRib were added to stimulated lymphocytes simultaneously with [^{3}H]TdR on the third day of culture. No inhibition in [^{3}H]TdR uptake was found to occur.

In another experiment, the DNA content of stimulated lymphocyte cultures which received Rib, dRib, Ara, or medium alone was evaluated at the end of the third day of culture. As shown in Table III, cultures which received the higher doses of Rib and dRib had not multiplied their DNA content.

(6) Time-course of the sugar action. To evaluate the action of Rib and dRib during the complex events leading to PHA-induced lymphocyte blastization and division, sugars

TABLE II. EFFECT OF Rib AND dRib ON DNA, RNA, AND PROTEIN SYNTHESIS IN PHA-STIMULATED LYMPHOCYTES

Sugar added at the beginning of the 72-hr culture	% Inhibition of incorporation		
	[^{3}H]TdR	[^{3}H]U	[^{14}C]Leu
None	—	—	—
30 m*M* Rib	69	63	76
50 m*M* Rib	97	73	99
2 m*M* dRib	41	46	72
10 m*M* dRib	99	89	100
30 m*M* Ara	10	10	18
50 m*M* Ara	20	N.P.	N.P.

Note. Results are averages of three independent experiments. SEM are not reported, since never exceeded 10.

were added to PHA-containing medium at the onset of the culture, then the medium was substituted in the wells after 24, 48, or 66 hr with a PHA-containing sugar-free one. Cultures were sacrificed as usual after a 6-hr pulse at the end of the 3rd day. Results are shown in Fig. 5. The presence of 10 m*M* dRib during the first 24 hr of stimulation is able to prevent the blastization of lymphocytes, while 2 m*M* dRib and Rib are required to be present for a longer time (48 hr) for the inhibition to take place.

(7) Contemporary action of Rib and dRib. Since the inhibition exerted by the two sugars is proportional to their concentrations, we can hypothesize that, should they influence the same chain of reactions, their inhibitory activities would sum up. In fact, the experiment presented in Table IV, in which the sugars were added at the onset of a PHA-stimulated culture, shows that the single inhibitions exerted by low concentrations of Rib and dRib sum up almost arithmetically when the two penthoses are added at the same time.

Discussion. Literature data on the effects of *in vitro* Rib and dRib addition are few and contradictory. Rib and dRib have been shown to inhibit IL-2-stimulated thymocyte proliferation at concentrations similar to those reported here (17). As far as Rib is concerned, it has also been found to inhibit mitogen-stimulated lymphocyte proliferation and mixed lymphocyte cultures (11); MacDermott *et al.* (9) have reported an inhibition of cell-mediated cytotoxicity with 25 and 50 m*M* Rib, but Kornbluth *et al.* (12), in a similar experimental system, found Rib ineffective as an inhibitor even at a concentration of 100 m*M*.

In this paper we have demonstrated that the inhibition was independent of the mitogenic stimulus applied to lymphocytes, of the lymphocyte subpopulation, i.e., T vs B cells, and of the lymphocyte origin, i.e., human vs murine; murine lymphocytes were inhibited by smaller concentrations of both sugars.

The results obtained using three established cell lines grown in liquid culture and normal bone marrow-derived fibroblasts demonstrated that both sugars also inhibit TdR incorporation in other cells apart from lymphocytes, thus indicating that they interfered with metabolic processes that are common to most cells.

Moreover, RNA and protein labeling experiments demonstrated that both sugars were able to inhibit the synthesis of these macromolecules as well as that of DNA. The hypothesis that a block of lymphocyte blastization had occurred is reinforced by the direct assessment of the DNA content in Rib and dRib-treated cells. Purity controls seem to rule out trivial explanations, i.e., the possibility that these inhibitions were due to contaminants in the sugars.

Morphological examination of unstimulated lymphocytes and of semiconfluent fibroblasts has shown that these nondividing cells die when treated with the two sugars, but that Rib and dRib are slow killers, since the effect

TABLE III. DNA CONTENT OF LYMPHOCYTE CULTURES TREATED WITH Rib AND dRib

	od (260 nm)
Control 1: unstimulated untreated lymphocytes	0.155
Control 2: stimulated untreated lymphocytes	0.360
Stimulated lymphocytes + 30 m*M* Rib	0.238
Stimulated lymphocytes + 50 m*M* Rib	0.168
Stimulated lymphocytes + 2 m*M* dRib	0.276
Stimulated lymphocytes + 10 m*M* dRib	0.127
Stimulated lymphocytes + 30 m*M* Ara	0.334
Stimulated lymphocytes + 50 m*M* Ara	0.360

Note. All cultures were set up with 10^6 cells and allowed to multiply 72 hr in the presence of PHA and of the desired concentrations of the sugars. The experiment was repeated twice with similar results.

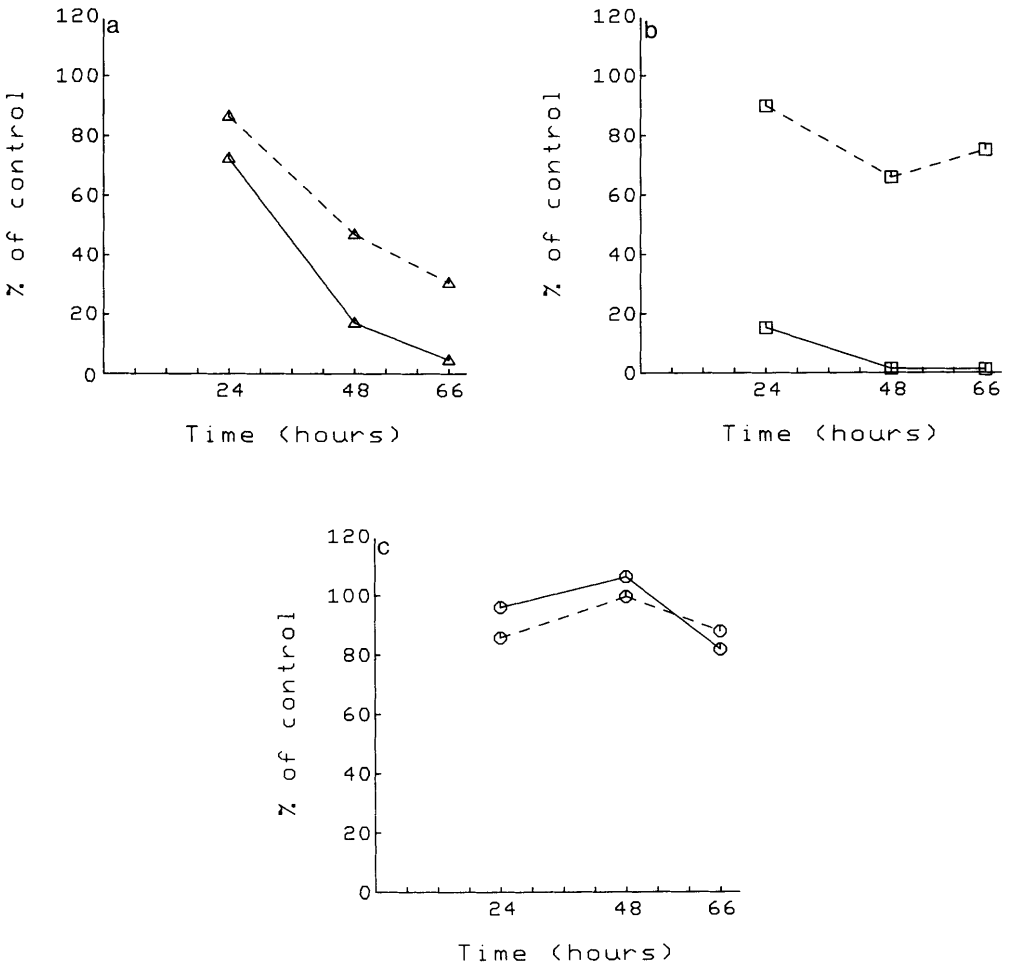

FIG. 5. [^{3}H]TdR incorporation of normal human lymphocytes stimulated with PHA and treated with sugars. The sugars were removed at the desired times and cultures were carried on till the end of the 72-hr cultures. Controls did not receive any sugar. Data are the average of triplicate wells of one experiment. The experiment was repeated once with similar results. (a) △ — △, 50 m*M* Rib; △ --- △, 30 m*M* Rib. (b) □ — □, 10 m*M* dRib; □ --- □, 2 m*M* dRib. (c) ○ — ○, 50 m*M* Ara; ○ --- ○, 30 m*M* Ara.

is assessable only 72 hr after their administration and is massive at the 96th hr. This delayed mortality does not mean, however, that subtler effects do not occur earlier, as demonstrated by the fact that unstimulated lymphocytes pretreated for 24–48 hr only with the sugars do not recover. The same time-course is also observable in PHA-stimulated lymphocytes where the presence of the two sugars during only the first 24–48 hr of culture, before most DNA synthesis starts taking place, leads to the block of subsequent cellular processes.

In spite of their capacity to kill various type of cells from different species *in vitro,* both Rib and dRib are apparently devoid of *in vivo* toxicity when administered for several days to mice at those concentrations that are inhibitory *in vitro* (data not shown).

It is worth noting that dRib is about five times more toxic than Rib in all experimental conditions tested. Whether or not Rib and dRib act on the same cellular substrate(s), as suggested by the experiment in which both sugars are administered at the same time to lymphocyte cultures, remains an unsettled question. Taking into account the metabolic pathways of the two sugars and the possibility that cells may convert dRib-5-P into Rib-5-P

TABLE IV. EFFECT OF THE CONTEMPORARY ADDITION OF Rib AND dRib ON THE [^{3}H]TdR INCORPORATION OF PHA-STIMULATED LYMPHOCYTES

Addition	[^{3}H]TdR (cpm)	% Inhibition	Sum of the single inhibitions (%)
None	44,468	—	—
0.5 m*M* dRib	39,205	12	—
1 m*M* dRib	27,978	37	—
2 m*M* dRib	25,984	42	—
10 m*M* Rib	39,553	11	—
20 m*M* Rib	30,622	31	—
0.5 m*M* dRib + 10 m*M* Rib	33,887	24	12 + 11 = 23
0.5 m*M* dRib + 20 m*M* Rib	26,926	39	12 + 31 = 43
1 m*M* dRib + 10 m*M* Rib	25,476	43	37 + 11 = 48
1 m*M* dRib + 20 m*M* Rib	21,891	51	37 + 31 = 68
2 m*M* dRib + 10 m*M* Rib	6,416	86	42 + 11 = 53
2 m*M* dRib + 20 m*M* Rib	574	99	42 + 31 = 73

Note. The sugars were added at the beginning of the 72-hr cultures. Values are the average of triplicate samples.

(22), the fivefold higher toxicity of dRib to Rib is even more intriguing.

At present, it is difficult to make an hypothesis concerning the physiological significance of these phenomena and the mechanisms by which the cytotoxicity of Rib and dRib can be explained at the biochemical level. Only Stankowa *et al.* (11) have offered an explanation for Rib-mediated inhibition, suggesting that Rib may be a competitive inhibitor of TdR incorporation. In the present paper we have demonstrated that when Rib and dRib were administered simultaneously with TdR, no inhibition could be observed; when the sugars were removed just before the addition of the label, the extent of the inhibition was unchanged: these findings rule out the hypothesis put forth by Stankowa *et al.* (11).

On the whole, our observations suggest that, unlike other simple sugars that, at the same concentrations, have negligible effects on cell metabolism (13, 17), Rib and dRib cause a deep derangement of key cell metabolism. Among the possible targets of these sugars, purine and pyrimidine metabolism (23) and ATP availability (24) should be considered. Further studies are needed to test these hypotheses.

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